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(54) Title: TUMOR-ASSOCIATED ANTIGEN			
<pre> 1  MCTGKCARCVGLSLITLCLVCIVANALLLYPNGETSWTNT 1634851 1  MCYIGKCARCIGHSLVGLALLCIANALLLYFPNGETKYASE GI 186804 1  MCYVVKCAFYIGYSLVWAAVFCIVANALLLYFPNGETKYATE GI 476343  41  NHLSLQVWLMGGFIFGGGLMVLCPGIAAVRAGGKGCAGC 1634851 41  NHLSRFVWFVFGIVGGGLMLLLEAFVFIGLEQDDCCG--C GI 186804 41  DHLSRFVWFVYFAGIVGGGLMLLLEAFVFIGMDEEDCCG--C GI 476343  81  CG-----NRCRMLRSVFPSSAFQVVGAIYCLSVSGAGLRNG 1634851 79  CGHENCGRKANLSSVLAALIGIAGSGYCVIVAALGLAEG GI 186804 79  CGYENYGRCSMLSSVLAALIGIVGSAYCVIVASLGLAEG GI 476343  116  PRCL-MNGEWGYHFDTAGAYLLNRTLWDRCEAPPRVVPW 1634851 119  FLCIDSLGQWNYTFASTEGQYLLDTSTWSECTEPKHIVEW GI 186804 119  FRKSDAHGVWNTTFASTEGQYLLNSSMWSKCYERKHIVEW GI 476343  155  NVTLFSLLVAAASCLIEIVLCGIQLYNATIGVFCGDC-RKKQ 1634851 159  NVSLFSLILALGGIEFILCLIQVINGVLGGICGFCSSHQQ GI 186804 159  HVTLFSLILAFAAVEFILCLIQVINGMLGGLCGYCSRQ GI 476343  194  DTPH 1634851 199  QYDC GI 186804 199  QYNC GI 476343 </pre>			
(57) Abstract			
<p>The present invention provides a human tumor-associated antigen (TUAN) and polynucleotides which identify and encode TUAN. The antigen is related to the transmembrane 4, super family (TM4SF) and is similar to L6 antigen. The invention also provides expression vectors, host cells, agonists, antibodies and antagonists. In addition, the invention provides methods for producing TUAN and for treating or preventing disorders associated with expression of TUAN.</p>			

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## TUMOR-ASSOCIATED ANTIGEN

### TECHNICAL FIELD

This invention relates to nucleic acid and amino acid sequences of a new tumor-associated antigen and to the use of these sequences in the diagnosis, prevention, and treatment of  
5 inflammation and disorders associated with cell proliferation.

### BACKGROUND ART

Cancers, or malignant tumors, which are characterized by continuous cell proliferation and cell death, can be classified into three categories: carcinomas, sarcomas, and leukemia.

Recent reports show that approximately one in eight women contracts breast cancer and that the  
10 risk of prostate cancer is about 9.5% among men over 50 years of age (Helzlsouer, K. J. (1994) Curr. Opin. Oncol. 6:541-548; Harris, J. R. et al. (1992) N. Engl. J. Med. 327:319-328). Cancer cells have been shown to exhibit unique gene expression, and dozens of cancer-specific genetic markers, tumor antigens, have been identified.

Tumor antigens are surface molecules that are differentially expressed in tumor cells  
15 relative to non-tumor tissues. Tumor antigens make tumor cells immunologically distinct from normal cells and provide diagnostic and therapeutic targets for human cancers. Several monoclonal antibodies have been identified which react specifically with cancerous cells such as T-cell acute lymphoblastic leukemia and neuroblastoma (Minegishi, M. et al. (1989) Leukemia Res. 13:43-51; Takagi, S. et al. (1995) Int. J. Cancer 61:706-715). In addition, the discovery of  
20 high level expression of the HER2 gene in breast tumors has led to the development of therapeutic treatments (Liu, E. et al. (1992) Oncogene 7:1027-1032; Kern, J. A. (1993) Am. J. Respir. Cell Mol. Biol. 9:448-454).

Tumor antigens have been characterized either as membrane proteins or as altered carbohydrate molecules of glycoproteins or glycolipids on the cell surface. A multigene family  
25 encoding type III integral membrane proteins which traverse the cell membrane four times has been identified (Wright, M.D. and Tomlinson, M.G. (1994) Immunol. Today 15:588-94). The transmembrane 4 superfamily (TM4SF) proteins are found predominantly in cells of hematopoietic origin and in tumors and include a number of platelet and endothelial cell membrane proteins, CD9 (lung adenocarcinoma antigen MRP-1), the platelet and melanoma-  
30 associated antigen CD63, leukocyte surface glycoproteins, CD53, CD37, CD63, and R2, the tumor associated antigen TAPA-1 (CD81), the colonal carcinoma antigen CO-029, mink lung epithelial protein TI-1, the tumor-associated antigens L6 and SAS, a gene amplified in human sarcomas (Wright and Tomlinson, supra; Jankowski, S.A. et al. (1994) Oncogene 9:1205-11).

These proteins share 25-30% amino acid sequence identity.

In the TM4SF proteins, the N- and C-termini are intracellular and the major hydrophilic domain, located between transmembrane domains 3 and 4, is extracellular. TM4SF proteins are most conserved in their transmembrane and cytoplasmic domains and most divergent in their hydrophilic extracellular domains which contain N-linked glycosylation sites. The high level of conservation in the transmembrane and cytoplasmic domains suggests an effector/signaling function. The divergence of the extracellular domains suggests that these hydrophilic domains provide functions specific to each protein such as ligand binding or protein-protein interaction (Wright and Tomlinson, *supra*).

10 A number of TM4SF proteins have been implicated in signal transduction, control of cell adhesion, and regulation of cell growth and proliferation (Wright and Tomlinson, *supra*; Jankowski, *supra*). Expression of some TM4SF proteins is associated with a variety of tumors and is altered when cells are activated or dividing. Other TM4SF proteins are implicated in cell growth due to their association with tumor cells. For example, CD9, CD53, and CD82 are  
15 upregulated when lymphocytes are activated while the expression of CD37 is abolished when B cells are activated. Although CD9 is not expressed on resting B and T lymphocytes, it is a marker for 90% of non-T acute lymphoblastic leukemia cells and for 50% of acute myeloid and chronic lymphoid leukemias. Anti-CD9 antibodies inhibit the motility of a variety of cancer cell lines and inhibit the metastatic potential of the mouse BL6 cell line (Miyake, M. and Hakomori,  
20 S. (1991) *Biochem.* 30:3328-34). Similarly, CD63 is not expressed on normal tissue melanocytes, but it is expressed in early stage melanoma. Another member of the TM4SF superfamily, the L6 surface antigen, is differentially expressed on lung, breast, colon, and ovarian carcinomas (Marken, J. S. et al. (1992) *Proc. Natl. Acad. Sci. USA* 89: 3503-3507; Marken, J. S. et al. (1994) *J. Biol. Chem.* 269: 7397-7401).

25 The discovery of a tumor-associated antigen similar to the tumor-associated L6 antigen and the polynucleotides which encode it satisfies a need in the art by providing new compositions which are useful in diagnosing, preventing, and treating inflammation and disorders associated with cell proliferation.

#### DISCLOSURE OF THE INVENTION

30 The present invention features a new tumor-associated antigen hereinafter designated TUAN and characterized as having similarity to a human L6 antigen and a mouse L6 antigen.

Accordingly, the invention features a substantially purified TUAN having the amino acid sequence shown in SEQ ID NO:1.

One aspect of the invention features an isolated and substantially purified polynucleotide that encodes TUAN. In a particular aspect, the polynucleotide is the nucleotide sequence of SEQ ID NO:2.

The invention also relates to a polynucleotide sequence comprising the complement of 5 SEQ ID NO:2 or variants thereof. In addition, the invention features polynucleotide sequences which hybridize under stringent conditions to SEQ ID NO:2.

The invention additionally features nucleic acid sequences encoding fragments, or the complement of the polynucleotide sequences, as well as expression vectors and host cells comprising polynucleotides that encode TUAN. The present invention also features antibodies 10 which bind specifically to TUAN, and pharmaceutical compositions comprising substantially purified TUAN. The invention also features methods for stimulating cell proliferation using TUAN, and for treating or preventing inflammation or a disorder associated with cell proliferation using an antagonist of TUAN.

#### BRIEF DESCRIPTION OF DRAWINGS

15 Figures 1A and 1B show the amino acid sequence (SEQ ID NO:1) and nucleic acid sequence (SEQ ID NO:2) of TUAN. The alignment was produced using MacDNASIS PRO™ software (Hitachi Software Engineering Co., Ltd., San Bruno, CA).

Figure 2 shows the amino acid sequence alignments among TUAN (SEQ ID NO:1), a human tumor-associated antigen, L6 (GI 186804; SEQ ID NO:3), and a mouse L6 antigen (GI 20 476343; SEQ ID NO:4). The alignment was produced using the multisequence alignment program of DNASTAR™ software (DNASTAR Inc, Madison WI).

Figures 3A and 3B show the hydrophobicity plots (MacDNASIS PRO software) for TUAN (SEQ ID NO: 1) and the human L6 antigen (SEQ ID NO:3), respectively. The positive X axis reflects amino acid position, and the negative Y axis, hydrophobicity.

#### 25 MODES FOR CARRYING OUT THE INVENTION

Before the present proteins, nucleotide sequences, and methods are described, it is understood that this invention is not limited to the particular methodology, protocols, cell lines, vectors, and reagents described as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to 30 limit the scope of the present invention which will be limited only by the appended claims.

It must be noted that as used herein and in the appended claims, the singular forms "a", "an", and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, reference to "a host cell" includes a plurality of such host cells, reference to the

"antibody" is a reference to one or more antibodies and equivalents thereof known to those skilled in the art, and so forth.

Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods, devices, and materials are now described. All publications mentioned herein are incorporated herein by reference for the purpose of describing and disclosing the cell lines, vectors, and methodologies which are reported in the publications which might be used in connection with the invention.

10 Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

#### DEFINITIONS

"Nucleic acid sequence" as used herein refers to an oligonucleotide, nucleotide, or polynucleotide, and fragments or portions thereof, and to DNA or RNA of genomic or synthetic origin which may be single- or double-stranded, and represent the sense or antisense strand.

15 Similarly, "amino acid sequence" as used herein refers to an oligopeptide, peptide, polypeptide, or protein sequence, and fragments or portions thereof, and to naturally occurring or synthetic molecules.

Where "amino acid sequence" is recited herein to refer to an amino acid sequence of a naturally occurring protein molecule, "amino acid sequence" and like terms, such as "polypeptide" or "protein" are not meant to limit the amino acid sequence to the complete, native amino acid sequence associated with the recited protein molecule.

"Peptide nucleic acid", as used herein, refers to a molecule which comprises an oligomer to which an amino acid residue, such as lysine, and an amino group have been added. These small molecules, also designated anti-gene agents, stop transcript elongation by binding to their complementary strand of nucleic acid (Nielsen, P.E. et al. (1993) Anticancer Drug Des. 8:53-63).

25

TUAN, as used herein, refers to the amino acid sequences of substantially purified TUAN obtained from any species, particularly mammalian, including bovine, ovine, porcine, murine, equine, and preferably human, from any source whether natural, synthetic, semi-synthetic, or recombinant.

30

"Consensus", as used herein, refers to a nucleic acid sequence which has been resequenced to resolve uncalled bases, or which has been extended using XL-PCR™ (Perkin Elmer, Norwalk, CT) in the 5' and/or the 3' direction and resequenced, or which has been

assembled from the overlapping sequences of more than one Incyte clone using the GELVIEW™ Fragment Assembly system (GCG, Madison, WI), or which has been both extended and assembled.

A "variant" of TUAN, as used herein, refers to an amino acid sequence that is altered by one or more amino acids. The variant may have "conservative" changes, wherein a substituted amino acid has similar structural or chemical properties, e.g., replacement of leucine with isoleucine. More rarely, a variant may have "nonconservative" changes, e.g., replacement of a glycine with a tryptophan. Similar minor variations may also include amino acid deletions or insertions, or both. Guidance in determining which amino acid residues may be substituted, inserted, or deleted without abolishing biological or immunological activity may be found using computer programs well known in the art, for example, DNASTAR software.

A "deletion", as used herein, refers to a change in either amino acid or nucleotide sequence in which one or more amino acid or nucleotide residues, respectively, are absent.

An "insertion" or "addition", as used herein, refers to a change in an amino acid or nucleotide sequence resulting in the addition of one or more amino acid or nucleotide residues, respectively, as compared to the naturally occurring molecule.

A "substitution", as used herein, refers to the replacement of one or more amino acids or nucleotides by different amino acids or nucleotides, respectively.

The term "biologically active", as used herein, refers to a protein having structural, regulatory, or biochemical functions of a naturally occurring molecule. Likewise, "immunologically active" refers to the capability of the natural, recombinant, or synthetic TUAN, or any oligopeptide thereof, to induce a specific immune response in appropriate animals or cells and to bind with specific antibodies.

The term "agonist", as used herein, refers to a molecule which, when bound to TUAN, causes a change in TUAN which modulates the activity of TUAN. Agonists may include proteins, nucleic acids, carbohydrates, or any other molecules which bind to TUAN.

The terms "antagonist" or "inhibitor", as used herein, refer to a molecule which, when bound to TUAN, blocks or modulates the biological or immunological activity of TUAN. Antagonists and inhibitors may include proteins, nucleic acids, carbohydrates, or any other molecules which bind to TUAN.

The term "modulate", as used herein, refers to a change or an alteration in the biological activity of TUAN. Modulation may be an increase or a decrease in protein activity, a change in binding characteristics, or any other change in the biological, functional or immunological

properties of TUAN.

The term "mimetic", as used herein, refers to a molecule, the structure of which is developed from knowledge of the structure of TUAN or portions thereof and, as such, is able to effect some or all of the actions related to the human tumor-associated antigen.

5       The term "derivative", as used herein, refers to the chemical modification of a nucleic acid encoding TUAN or the encoded TUAN. Illustrative of such modifications would be replacement of hydrogen by an alkyl, acyl, or amino group. A nucleic acid derivative would encode a polypeptide which retains essential biological characteristics of the natural molecule.

10       The term "substantially purified", as used herein, refers to nucleic or amino acid sequences that are removed from their natural environment, isolated or separated, and are at least 60% free, preferably 75% free, and most preferably 90% free from other components with which they are naturally associated.

"Amplification" as used herein refers to the production of additional copies of a nucleic acid sequence and is generally carried out using polymerase chain reaction (PCR) technologies well known in the art (Dieffenbach, C.W. and G.S. Dveksler (1995) PCR Primer, a Laboratory  
15 Manual, Cold Spring Harbor Press, Plainview, NY).

The term "hybridization", as used herein, refers to any process by which a strand of nucleic acid binds with a complementary strand through base pairing.

20       The term "hybridization complex", as used herein, refers to a complex formed between two nucleic acid sequences by virtue of the formation of hydrogen bonds between complementary G and C bases and between complementary A and T bases; these hydrogen bonds may be further stabilized by base stacking interactions. The two complementary nucleic acid sequences hydrogen bond in an antiparallel configuration. A hybridization complex may be formed in solution (e.g., C<sub>0</sub>t or R<sub>0</sub>t analysis) or between one nucleic acid sequence present in solution and  
25 another nucleic acid sequence immobilized on a solid support (e.g., membranes, filters, chips, pins or glass slides to which cells have been fixed for *in situ* hybridization).

30       The terms "complementary" or "complementarity", as used herein, refer to the natural binding of polynucleotides under permissive salt and temperature conditions by base-pairing. For example, for the sequence "A-G-T" binds to the complementary sequence "T-C-A". Complementarity between two single-stranded molecules may be "partial", in which only some of the nucleic acids bind, or it may be complete when total complementarity exists between the single stranded molecules. The degree of complementarity between nucleic acid strands has significant effects on the efficiency and strength of hybridization between nucleic acid strands.



This is of particular importance in amplification reactions, which depend upon binding between nucleic acids strands.

The term "homology", as used herein, refers to a degree of complementarity. There may be partial homology or complete homology (i.e., identity). A partially complementary sequence is one that at least partially inhibits an identical sequence from hybridizing to a target nucleic acid; it is referred to using the functional term "substantially homologous." The inhibition of hybridization of the completely complementary sequence to the target sequence may be examined using a hybridization assay (Southern or northern blot, solution hybridization and the like) under conditions of low stringency. A substantially homologous sequence or probe will compete for and inhibit the binding (i.e., the hybridization) of a completely homologous sequence or probe to the target sequence under conditions of low stringency. This is not to say that conditions of low stringency are such that non-specific binding is permitted; low stringency conditions require that the binding of two sequences to one another be a specific (i.e., selective) interaction. The absence of non-specific binding may be tested by the use of a second target sequence which lacks even a partial degree of complementarity (e.g., less than about 30% identity); in the absence of non-specific binding, the probe will not hybridize to the second non-complementary target sequence.

As known in the art, numerous equivalent conditions may be employed to comprise either low or high stringency conditions. Factors such as the length and nature (DNA, RNA, base composition) of the sequence, nature of the target (DNA, RNA, base composition, presence in solution or immobilization, etc.), and the concentration of the salts and other components (e.g., the presence or absence of formamide, dextran sulfate and/or polyethylene glycol) are considered and the hybridization solution may be varied to generate conditions of either low or high stringency different from, but equivalent to, the above listed conditions.

The term "stringent conditions", as used herein, is the "stringency" which occurs within a range from about  $T_m - 5^\circ\text{C}$  ( $5^\circ\text{C}$  below the melting temperature ( $T_m$ ) of the probe) to about  $20^\circ\text{C}$  to  $25^\circ\text{C}$  below  $T_m$ . As will be understood by those of skill in the art, the stringency of hybridization may be altered in order to identify or detect identical or related polynucleotide sequences.

The term "antisense", as used herein, refers to nucleotide sequences which are complementary to a specific DNA or RNA sequence. The term "antisense strand" is used in reference to a nucleic acid strand that is complementary to the "sense" strand. Antisense molecules may be produced by any method, including synthesis by ligating the gene(s) of interest

in a reverse orientation to a viral promoter which permits the synthesis of a complementary strand. Once introduced into a cell, this transcribed strand combines with natural sequences produced by the cell to form duplexes. These duplexes then block either the further transcription or translation. In this manner, mutant phenotypes may be generated. The designation "negative" is sometimes used in reference to the antisense strand, and "positive" is sometimes used in reference to the sense strand.

The term "portion", as used herein, with regard to a protein (as in "a portion of a given protein") refers to fragments of that protein. The fragments may range in size from four amino acid residues to the entire amino acid sequence minus one amino acid. Thus, a protein comprising at least a portion of the amino acid sequence of SEQ ID NO:1" encompasses the full-length human TUAN and fragments thereof.

"Transformation", as defined herein, describes a process by which exogenous DNA enters and changes a recipient cell. It may occur under natural or artificial conditions using various methods well known in the art. Transformation may rely on any known method for the insertion of foreign nucleic acid sequences into a prokaryotic or eukaryotic host cell. The method is selected based on the host cell being transformed and may include, but is not limited to, viral infection, electroporation, lipofection, and particle bombardment. Such "transformed" cells include stably transformed cells in which the inserted DNA is capable of replication either as an autonomously replicating plasmid or as part of the host chromosome. They also include cells which transiently express the inserted DNA or RNA for limited periods of time.

The term "antigenic determinant", as used herein, refers to that portion of a molecule that makes contact with a particular antibody (i.e., an epitope). When a protein or fragment of a protein is used to immunize a host animal, numerous regions of the protein may induce the production of antibodies which bind specifically to a given region or three-dimensional structure on the protein; these regions or structures are referred to as antigenic determinants. An antigenic determinant may compete with the intact antigen (i.e., the immunogen used to elicit the immune response) for binding to an antibody.

The terms "specific binding" or "specifically binding", as used herein, in reference to the interaction of an antibody and a protein or peptide, mean that the interaction is dependent upon the presence of a particular structure (i.e., the antigenic determinant or epitope) on the protein; in other words, the antibody is recognizing and binding to a specific protein structure rather than to proteins in general. For example, if an antibody is specific for epitope "A", the presence of a protein containing epitope A (or free, unlabeled A) in a reaction containing labeled "A" and the

antibody will reduce the amount of labeled A bound to the antibody.

The term "sample", as used herein, is used in its broadest sense. A biological sample suspected of containing nucleic acid encoding TUAN or fragments thereof may comprise a cell, chromosomes isolated from a cell (e.g., a spread of metaphase chromosomes), genomic DNA (in solution or bound to a solid support such as for Southern analysis), RNA (in solution or bound to a solid support such as for northern analysis), cDNA (in solution or bound to a solid support), an extract from cells or a tissue, and the like.

The term "correlates with expression of a polynucleotide", as used herein, indicates that the detection of the presence of ribonucleic acid that is similar to SEQ ID NO:2 by northern analysis is indicative of the presence of mRNA encoding TUAN in a sample and thereby correlates with expression of the transcript from the polynucleotide encoding the protein.

"Alterations" in the polynucleotide of SEQ ID NO: 2, as used herein, comprise any alteration in the sequence of polynucleotides encoding TUAN including deletions, insertions, and point mutations that may be detected using hybridization assays. Included within this definition is the detection of alterations to the genomic DNA sequence which encodes TUAN (e.g., by alterations in the pattern of restriction fragment length polymorphisms capable of hybridizing to SEQ ID NO:2), the inability of a selected fragment of SEQ ID NO: 2 to hybridize to a sample of genomic DNA (e.g., using allele-specific oligonucleotide probes), and improper or unexpected hybridization, such as hybridization to a locus other than the normal chromosomal locus for the polynucleotide sequence encoding TUAN (e.g., using fluorescent *in situ* hybridization [FISH] to metaphase chromosomes spreads).

As used herein, the term "antibody" refers to intact molecules as well as fragments thereof, such as Fa, F(ab')<sub>2</sub>, and Fv, which are capable of binding the epitopic determinant. Antibodies that bind TUAN polypeptides can be prepared using intact polypeptides or fragments containing small peptides of interest as the immunizing antigen. The polypeptide or peptide used to immunize an animal can be derived from the translation of RNA or synthesized chemically, and can be conjugated to a carrier protein, if desired. Commonly used carriers that are chemically coupled to peptides include bovine serum albumin and thyroglobulin. The coupled peptide is then used to immunize the animal (e.g., a mouse, a rat, or a rabbit).

The term "humanized antibody", as used herein, refers to antibody molecules in which amino acids have been replaced in the non-antigen binding regions in order to more closely resemble a human antibody, while still retaining the original binding ability.

**THE INVENTION**

The invention is based on the discovery of a new human tumor-associated antigen (TUAN), the polynucleotides encoding TUAN, and the use of these compositions for the diagnosis, prevention, or treatment of inflammation and disorders associated with cell proliferation.

Nucleic acids encoding the human TUAN of the present invention were first identified in Incyte Clone 1634851 from an colon tissue cDNA library (COLNNOT19) through a computer search for amino acid sequence alignments. SEQ ID NO:2 was derived by extension of Incyte Clone 1634851.

10 In one embodiment, the invention encompasses a polypeptide comprising the amino acid sequence of SEQ ID NO:1, as shown in Figures 1A and 1B. TUAN is 197 amino acids in length. It has four potential transmembrane domains encompassing residues L14-V30, F53-A70, V91-L112, and L162-189, analogous to other TM4SF proteins. TUAN also has seven conserved cysteine residues at C75, C76, C80, C81, C85, C118, and C145, identical to the human and  
15 mouse L6 antigens (GI 186804 and GI 476343). In addition, TUAN has three potential N-glycosylation sites at N138-L141, N155-L158, and N179-I182, two casein kinase II phosphorylation sites at T140-D143 and S166-E169, and one potential protein kinase C phosphorylation site at T3-K5. As shown in Figure 2, TUAN has chemical and structural  
20 homology with a human L6 antigen, CD53 (GI 186804; SEQ ID NO:3) and a mouse L6 antigen (GI 476343; SEQ ID NO:4). In particular, TUAN shares 47% identity with the human L6 antigen and 45% identity with the mouse L6 antigen. As illustrated by Figures 3A and 3B, TUAN and the human L6 antigen have rather similar hydrophobicity plots. Northern analysis shows the expression of TUAN in various cDNA libraries, all of which are characterized by cell division; at least 20% of which are immortalized or cancerous, at least 20% of which involve immune  
25 response, and at least 60% of which involve fetal/infant tissues or organs.

The invention also encompasses TUAN variants which retain biological or other functional activity of TUAN. A preferred TUAN variant is one having at least 80%, and more preferably 90%, amino acid sequence identity to the TUAN amino acid sequence (SEQ ID NO:1). A most preferred TUAN variant is one having at least 95% amino acid sequence identity to SEQ  
30 ID NO:1.

The invention also encompasses polynucleotides which encode TUAN. Accordingly, any nucleic acid sequence which encodes the amino acid sequence of TUAN can be used to generate recombinant molecules which express TUAN. In a particular embodiment, the invention

encompasses the polynucleotide comprising the nucleic acid sequence of SEQ ID NO:2 as shown in Figures 1A and 1B.

It will be appreciated by those skilled in the art that as a result of the degeneracy of the genetic code, a multitude of nucleotide sequences encoding TUAN, some bearing minimal  
5 homology to the nucleotide sequences of any known and naturally occurring gene, may be produced. Thus, the invention contemplates each and every possible variation of nucleotide sequence that could be made by selecting combinations based on possible codon choices. These combinations are made in accordance with the standard triplet genetic code as applied to the nucleotide sequence of naturally occurring TUAN, and all such variations are to be considered as  
10 being specifically disclosed.

Although nucleotide sequences which encode TUAN and its variants are preferably capable of hybridizing to the nucleotide sequence of the naturally occurring TUAN under appropriately selected conditions of stringency, it may be advantageous to produce nucleotide sequences encoding TUAN or its derivatives possessing a substantially different codon usage.  
15 Codons may be selected to increase the rate at which expression of the peptide occurs in a particular prokaryotic or eukaryotic host in accordance with the frequency with which particular codons are utilized by the host. Other reasons for substantially altering the nucleotide sequence encoding TUAN and its derivatives without altering the encoded amino acid sequences include the production of RNA transcripts having more desirable properties, such as a greater half-life,  
20 than transcripts produced from the naturally occurring sequence.

The invention also encompasses production of DNA sequences, or portions thereof, which encode TUAN and its derivatives, entirely by synthetic chemistry. After production, the synthetic sequence may be inserted into any of the many available expression vectors and cell systems using reagents that are well known in the art at the time of the filing of this application.  
25 Moreover, synthetic chemistry may be used to introduce mutations into a sequence encoding TUAN or any portion thereof.

Also encompassed by the invention are polynucleotide sequences that are capable of hybridizing to the claimed nucleotide sequences, and in particular, those shown in SEQ ID NO:2, under various conditions of stringency. Hybridization conditions are based on the melting  
30 temperature ( $T_m$ ) of the nucleic acid binding complex or probe, as taught in Wahl, G.M. and S.L. Berger (1987; Methods Enzymol. 152:399-407) and Kimmel, A.R. (1987; Methods Enzymol. 152:507-511), and may be used at a defined stringency.

Altered nucleic acid sequences encoding TUAN which are encompassed by the invention

include deletions, insertions, or substitutions of different nucleotides resulting in a polynucleotide that encodes the same or a functionally equivalent TUAN. The encoded protein may also contain deletions, insertions, or substitutions of amino acid residues which produce a silent change and result in a functionally equivalent TUAN. Deliberate amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues as long as the biological activity of TUAN is retained. For example, negatively charged amino acids may include aspartic acid and glutamic acid; positively charged amino acids may include lysine and arginine; and amino acids with uncharged polar head groups having similar hydrophilicity values may include leucine, isoleucine, and valine; glycine and alanine; asparagine and glutamine; serine and threonine; phenylalanine and tyrosine.

Also included within the scope of the present invention are alleles of the genes encoding TUAN. As used herein, an "allele" or "allelic sequence" is an alternative form of the gene which may result from at least one mutation in the nucleic acid sequence. Alleles may result in altered mRNAs or polypeptides whose structure or function may or may not be altered. Any given gene may have none, one, or many allelic forms. Common mutational changes which give rise to alleles are generally ascribed to natural deletions, additions, or substitutions of nucleotides. Each of these types of changes may occur alone, or in combination with the others, one or more times in a given sequence.

Methods for DNA sequencing which are well known and generally available in the art may be used to practice any embodiments of the invention. The methods may employ such enzymes as the Klenow fragment of DNA polymerase I, Sequenase® (US Biochemical Corp, Cleveland, OH), Taq polymerase (Perkin Elmer), thermostable T7 polymerase (Amersham, Chicago, IL), or combinations of recombinant polymerases and proofreading exonucleases such as the ELONGASE Amplification System marketed by Gibco BRL (Gaithersburg, MD). Preferably, the process is automated with machines such as the Hamilton Micro Lab 2200 (Hamilton, Reno, NV), Peltier Thermal Cycler (PTC200; MJ Research, Watertown, MA) and the ABI 377 DNA sequencers (Perkin Elmer).

The nucleic acid sequences encoding TUAN may be extended utilizing a partial nucleotide sequence and employing various methods known in the art to detect upstream sequences such as promoters and regulatory elements. For example, one method which may be employed, "restriction-site" PCR, uses universal primers to retrieve unknown sequence adjacent to a known locus (Sarkar, G. (1993) PCR Methods Applic. 2:318-322). In particular, genomic DNA is first amplified in the presence of primer to linker sequence and a primer specific to the

known region. The amplified sequences are then subjected to a second round of PCR with the same linker primer and another specific primer internal to the first one. Products of each round of PCR are transcribed with an appropriate RNA polymerase and sequenced using reverse transcriptase.

5 Inverse PCR may also be used to amplify or extend sequences using divergent primers based on a known region (Triglia, T. et al. (1988) *Nucleic Acids Res.* 16:8186). The primers may be designed using OLIGO 4.06 Primer Analysis software (National Biosciences Inc., Plymouth, MN), or another appropriate program, to be 22-30 nucleotides in length, to have a GC content of 50% or more, and to anneal to the target sequence at temperatures about 68°-72° C. The method  
10 uses several restriction enzymes to generate a suitable fragment in the known region of a gene. The fragment is then circularized by intramolecular ligation and used as a PCR template.

Another method which may be used is capture PCR which involves PCR amplification of DNA fragments adjacent to a known sequence in human and yeast artificial chromosome DNA (Lagerstrom, M. et al. (1991) *PCR Methods Applic.* 1:111-119). In this method, multiple  
15 restriction enzyme digestions and ligations may also be used to place an engineered double-stranded sequence into an unknown portion of the DNA molecule before performing PCR.

Another method which may be used to retrieve unknown sequences is that of Parker, J.D. et al. (1991; *Nucleic Acids Res.* 19:3055-3060). Additionally, one may use PCR, nested primers,  
20 and PromoterFinder™ libraries to walk in genomic DNA (Clontech, Palo Alto, CA). This process avoids the need to screen libraries and is useful in finding intron/exon junctions.

When screening for full-length cDNAs, it is preferable to use libraries that have been size-selected to include larger cDNAs. Also, random-primed libraries are preferable, in that they will contain more sequences which contain the 5' regions of genes. Use of a randomly primed  
25 library may be especially preferable for situations in which an oligo d(T) library does not yield a full-length cDNA. Genomic libraries may be useful for extension of sequence into the 5' and 3' non-transcribed regulatory regions.

Capillary electrophoresis systems which are commercially available may be used to analyze the size or confirm the nucleotide sequence of sequencing or PCR products. In  
30 particular, capillary sequencing may employ flowable polymers for electrophoretic separation, four different fluorescent dyes (one for each nucleotide) which are laser activated, and detection of the emitted wavelengths by a charge coupled device camera. Output/light intensity may be converted to electrical signal using appropriate software (e.g. Genotyper™ and Sequence

Navigator™, Perkin Elmer) and the entire process from loading of samples to computer analysis and electronic data display may be computer controlled. Capillary electrophoresis is especially preferable for the sequencing of small pieces of DNA which might be present in limited amounts in a particular sample.

5 In another embodiment of the invention, polynucleotide sequences or fragments thereof which encode TUAN, or fusion proteins or functional equivalents thereof, may be used in recombinant DNA molecules to direct expression of TUAN in appropriate host cells. Due to the inherent degeneracy of the genetic code, other DNA sequences which encode substantially the same or a functionally equivalent amino acid sequence may be produced and these sequences  
10 may be used to clone and express TUAN.

As will be understood by those of skill in the art, it may be advantageous to produce TUAN-encoding nucleotide sequences possessing non-naturally occurring codons. For example, codons preferred by a particular prokaryotic or eukaryotic host can be selected to increase the rate of protein expression or to produce a recombinant RNA transcript having desirable properties,  
15 such as a half-life which is longer than that of a transcript generated from the naturally occurring sequence.

The nucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter TUAN encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression  
20 of the gene product. DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. For example, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, or introduce mutations, and so forth.

In another embodiment of the invention, natural, modified, or recombinant nucleic acid  
25 sequences encoding TUAN may be ligated to a heterologous sequence to encode a fusion protein. For example, to screen peptide libraries for inhibitors of TUAN activity, it may be useful to encode a chimeric TUAN protein that can be recognized by a commercially available antibody. A fusion protein may also be engineered to contain a cleavage site located between the TUAN encoding sequence and the heterologous protein sequence, so that TUAN may be cleaved and  
30 purified away from the heterologous moiety.

In another embodiment, sequences encoding TUAN may be synthesized, in whole or in part, using chemical methods well known in the art (see Caruthers, M.H. et al. (1980) Nucl. Acids Res. Symp. Ser. 215-223, Horn, T. et al. (1980) Nucl. Acids Res. Symp. Ser. 225-232).



Alternatively, the protein itself may be produced using chemical methods to synthesize the amino acid sequence of TUAN, or a portion thereof. For example, peptide synthesis can be performed using various solid-phase techniques (Roberge, J.Y. et al. (1995) Science 269:202-204) and automated synthesis may be achieved, for example, using the ABI 431A Peptide Synthesizer  
5 (Perkin Elmer).

The newly synthesized peptide may be substantially purified by preparative high performance liquid chromatography (e.g., Creighton, T. (1983) Proteins, Structures and Molecular Principles, WH Freeman and Co., New York, NY). The composition of the synthetic peptides may be confirmed by amino acid analysis or sequencing (e.g., the Edman degradation  
10 procedure; Creighton, supra). Additionally, the amino acid sequence of TUAN, or any part thereof, may be altered during direct synthesis and/or combined using chemical methods with sequences from other proteins, or any part thereof, to produce a variant polypeptide.

In order to express a biologically active TUAN, the nucleotide sequences encoding TUAN or functional equivalents, may be inserted into appropriate expression vector, i.e., a vector which  
15 contains the necessary elements for the transcription and translation of the inserted coding sequence.

Methods which are well known to those skilled in the art may be used to construct expression vectors containing sequences encoding TUAN and appropriate transcriptional and translational control elements. These methods include in vitro recombinant DNA techniques,  
20 synthetic techniques, and in vivo genetic recombination. Such techniques are described in Sambrook, J. et al. (1989) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, Plainview, NY, and Ausubel, F.M. et al. (1989) Current Protocols in Molecular Biology, John Wiley & Sons, New York, NY.

A variety of expression vector/host systems may be utilized to contain and express  
25 sequences encoding TUAN. These include, but are not limited to, microorganisms such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems infected with virus expression vectors (e.g., baculovirus); plant cell systems transformed with virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or with bacterial  
30 expression vectors (e.g., Ti or pBR322 plasmids); or animal cell systems.

The "control elements" or "regulatory sequences" are those non-translated regions of the vector--enhancers, promoters, 5' and 3' untranslated regions--which interact with host cellular proteins to carry out transcription and translation. Such elements may vary in their strength and

specificity. Depending on the vector system and host utilized, any number of suitable transcription and translation elements, including constitutive and inducible promoters, may be used. For example, when cloning in bacterial systems, inducible promoters such as the hybrid lacZ promoter of the Bluescript® phagemid (Stratagene, LaJolla, CA) or pSport1™ plasmid 5 (Gibco BRL) and the like may be used. The baculovirus polyhedrin promoter may be used in insect cells. Promoters or enhancers derived from the genomes of plant cells (e.g., heat shock, RUBISCO; and storage protein genes) or from plant viruses (e.g., viral promoters or leader sequences) may be cloned into the vector. In mammalian cell systems, promoters from mammalian genes or from mammalian viruses are preferable. If it is necessary to generate a cell 10 line that contains multiple copies of the sequence encoding TUAN, vectors based on SV40 or EBV may be used with an appropriate selectable marker.

In bacterial systems, a number of expression vectors may be selected depending upon the use intended for TUAN. For example, when large quantities of TUAN are needed for the induction of antibodies, vectors which direct high level expression of fusion proteins that are 15 readily purified may be used. Such vectors include, but are not limited to, the multifunctional *E. coli* cloning and expression vectors such as Bluescript® (Stratagene), in which the sequence encoding TUAN may be ligated into the vector in frame with sequences for the amino-terminal Met and the subsequent 7 residues of  $\beta$ -galactosidase so that a hybrid protein is produced; pIN vectors (Van Heeke, G. and S.M. Schuster (1989) J. Biol. Chem. 264:5503-5509); and the like. 20 pGEX vectors (Promega, Madison, WI) may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. Proteins made in such systems may be designed to include heparin, thrombin, or factor XA protease cleavage sites so that the cloned polypeptide of 25 interest can be released from the GST moiety at will.

In the yeast, *Saccharomyces cerevisiae*, a number of vectors containing constitutive or inducible promoters such as alpha factor, alcohol oxidase, and PGH may be used. For reviews, see Ausubel et al. (supra) and Grant et al. (1987) Methods Enzymol. 153:516-544.

In cases where plant expression vectors are used, the expression of sequences encoding 30 TUAN may be driven by any of a number of promoters. For example, viral promoters such as the 35S and 19S promoters of CaMV may be used alone or in combination with the omega leader sequence from TMV (Takamatsu, N. (1987) EMBO J. 6:307-311). Alternatively, plant promoters such as the small subunit of RUBISCO or heat shock promoters may be used (Coruzzi,

G. et al. (1984) EMBO J. 3:1671-1680; Broglie, R. et al. (1984) Science 224:838-843; and Winter, J. et al. (1991) Results Probl. Cell Differ. 17:85-105). These constructs can be introduced into plant cells by direct DNA transformation or pathogen-mediated transfection. Such techniques are described in a number of generally available reviews (see, for example, 5 Hobbs, S. or Murry, L.E. in McGraw Hill Yearbook of Science and Technology (1992) McGraw Hill, New York, NY; pp. 191-196.

An insect system may also be used to express TUAN. For example, in one such system, Autographa californica nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes in Spodoptera frugiperda cells or in Trichoplusia larvae. The sequences encoding TUAN 10 may be cloned into a non-essential region of the virus, such as the polyhedrin gene, and placed under control of the polyhedrin promoter. Successful insertion of TUAN will render the polyhedrin gene inactive and produce recombinant virus lacking coat protein. The recombinant viruses may then be used to infect, for example, S. frugiperda cells or Trichoplusia larvae in which TUAN may be expressed (Engelhard, E.K. et al. (1994) Proc. Nat. Acad. Sci. 15 91:3224-3227).

In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, sequences encoding TUAN may be ligated into an adenovirus transcription/translation complex consisting of the late promoter and tripartite leader sequence. Insertion in a non-essential E1 or E3 region of the viral genome may 20 be used to obtain a viable virus which is capable of expressing TUAN in infected host cells (Logan, J. and Shenk, T. (1984) Proc. Natl. Acad. Sci. 81:3655-3659). In addition, transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, may be used to increase expression in mammalian host cells.

Specific initiation signals may also be used to achieve more efficient translation of 25 sequences encoding TUAN. Such signals include the ATG initiation codon and adjacent sequences. In cases where sequences encoding TUAN, its initiation codon, and upstream sequences are inserted into the appropriate expression vector, no additional transcriptional or translational control signals may be needed. However, in cases where only coding sequence, or a portion thereof, is inserted, exogenous translational control signals including the ATG initiation 30 codon should be provided. Furthermore, the initiation codon should be in the correct reading frame to ensure translation of the entire insert. Exogenous translational elements and initiation codons may be of various origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of enhancers which are appropriate for the particular cell system

which is used, such as those described in the literature (Scharf, D. et al. (1994) Results Probl. Cell Differ. 20:125-162).

In addition, a host cell strain may be chosen for its ability to modulate the expression of the inserted sequences or to process the expressed protein in the desired fashion. Such  
5 modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. Post-translational processing which cleaves a "prepro" form of the protein may also be used to facilitate correct insertion, folding and/or function. Different host cells such as CHO, HeLa, MDCK, HEK293, and WI38, which have specific cellular machinery and characteristic mechanisms for such post-translational  
10 activities, may be chosen to ensure the correct modification and processing of the foreign protein.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express TUAN may be transformed using expression vectors which may contain viral origins of replication and/or endogenous expression elements and a selectable marker gene on the same or on a separate vector. Following the  
15 introduction of the vector, cells may be allowed to grow for 1-2 days in an enriched media before they are switched to selective media. The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth and recovery of cells which successfully express the introduced sequences. Resistant clones of stably transformed cells may be proliferated using tissue culture techniques appropriate to the cell type.

20 Any number of selection systems may be used to recover transformed cell lines. These include, but are not limited to, the herpes simplex virus thymidine kinase (Wigler, M. et al. (1977) Cell 11:223-32) and adenine phosphoribosyltransferase (Lowy, I. et al. (1980) Cell 22:817-23) genes which can be employed in tk<sup>-</sup> or aprt<sup>-</sup> cells, respectively. Also, antimetabolite, antibiotic or herbicide resistance can be used as the basis for selection; for example, dhfr which  
25 confers resistance to methotrexate (Wigler, M. et al. (1980) Proc. Natl. Acad. Sci. 77:3567-70); npt, which confers resistance to the aminoglycosides neomycin and G-418 (Colbere-Garapin, F. et al (1981) J. Mol. Biol. 150:1-14) and als or pat, which confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively (Murry, supra). Additional selectable genes have been described, for example, trpB, which allows cells to utilize indole in place of tryptophan, or  
30 hisD, which allows cells to utilize histinol in place of histidine (Hartman, S.C. and R.C. Mulligan (1988) Proc. Natl. Acad. Sci. 85:8047-51). Recently, the use of visible markers has gained popularity with such markers as anthocyanins,  $\beta$  glucuronidase and its substrate GUS, and luciferase and its substrate luciferin, being widely used not only to identify transformants, but

also to quantify the amount of transient or stable protein expression attributable to a specific vector system (Rhodes, C.A. et al. (1995) *Methods Mol. Biol.* 55:121-131).

Although the presence/absence of marker gene expression suggests that the gene of interest is also present, its presence and expression may need to be confirmed. For example, if the sequence encoding TUAN is inserted within a marker gene sequence, recombinant cells containing sequences encoding TUAN can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with a sequence encoding TUAN under the control of a single promoter. Expression of the marker gene in response to induction or selection usually indicates expression of the tandem gene as well.

10 Alternatively, host cells which contain the nucleic acid sequence encoding TUAN and express TUAN may be identified by a variety of procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridizations and protein bioassay or immunoassay techniques which include membrane, solution, or chip based technologies for the detection and/or quantification of nucleic acid or protein.

15 The presence of polynucleotide sequences encoding TUAN can be detected by DNA-DNA or DNA-RNA hybridization or amplification using probes or portions or fragments of polynucleotides encoding TUAN. Nucleic acid amplification based assays involve the use of oligonucleotides or oligomers based on the sequences encoding TUAN to detect transformants containing DNA or RNA encoding TUAN. As used herein "oligonucleotides" or "oligomers"  
20 refer to a nucleic acid sequence of at least about 10 nucleotides and as many as about 60 nucleotides, preferably about 15 to 30 nucleotides, and more preferably about 20-25 nucleotides, which can be used as a probe or amplimer.

A variety of protocols for detecting and measuring the expression of TUAN, using either polyclonal or monoclonal antibodies specific for the protein are known in the art. Examples  
25 include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and fluorescence activated cell sorting (FACS). A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on TUAN is preferred, but a competitive binding assay may be employed. These and other assays are described, among other places, in Hampton, R. et al. (1990; Serological Methods, a Laboratory Manual, APS Press, St  
30 Paul, MN) and Maddox, D.E. et al. (1983; *J. Exp. Med.* 158:1211-1216).

A wide variety of labels and conjugation techniques are known by those skilled in the art and may be used in various nucleic acid and amino acid assays. Means for producing labeled hybridization or PCR probes for detecting sequences related to polynucleotides encoding TUAN

include oligolabeling, nick translation, end-labeling or PCR amplification using a labeled nucleotide. Alternatively, the sequences encoding TUAN, or any portions thereof may be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes in vitro by addition of an appropriate RNA polymerase such as T7, T3, or SP6 and labeled nucleotides. These procedures may be conducted using a variety of commercially available kits (Pharmacia & Upjohn, (Kalamazoo, MI); Promega (Madison WI); and U.S. Biochemical Corp., Cleveland, OH). Suitable reporter molecules or labels, which may be used, include radionuclides, enzymes, fluorescent, chemiluminescent, or chromogenic agents as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

Host cells transformed with nucleotide sequences encoding TUAN may be cultured under conditions suitable for the expression and recovery of the protein from cell culture. The protein produced by a recombinant cell may be secreted or contained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides which encode TUAN may be designed to contain signal sequences which direct secretion of TUAN through a prokaryotic or eukaryotic cell membrane. Other recombinant constructions may be used to join sequences encoding TUAN to nucleotide sequence encoding a polypeptide domain which will facilitate purification of soluble proteins. Such purification facilitating domains include, but are not limited to, metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilized metals, protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp., Seattle, WA). The inclusion of cleavable linker sequences such as those specific for Factor XA or enterokinase (Invitrogen, San Diego, CA) between the purification domain and TUAN may be used to facilitate purification. One such expression vector provides for expression of a fusion protein containing TUAN and a nucleic acid encoding 6 histidine residues preceding a thioredoxin or an enterokinase cleavage site. The histidine residues facilitate purification on IMIAC (immobilized metal ion affinity chromatography as described in Porath, J. et al. (1992, Prot. Exp. Purif. 3:263-281) while the enterokinase cleavage site provides a means for purifying TUAN from the fusion protein. A discussion of vectors which contain fusion proteins is provided in Kroll, D.J. et al. (1993; DNA Cell Biol. 12:441-453).

In addition to recombinant production, fragments of TUAN may be produced by direct peptide synthesis using solid-phase techniques Merrifield J. (1963) J. Am. Chem. Soc.

85:2149-2154). Protein synthesis may be performed using manual techniques or by automation. Automated synthesis may be achieved, for example, using Applied Biosystems 431A Peptide Synthesizer (Perkin Elmer). Various fragments of TUAN may be chemically synthesized separately and combined using chemical methods to produce the full length molecule.

## 5 THERAPEUTICS

TUAN shares chemical and structural homology with a human tumor-associated antigen, L6 (GI 186804) and a mouse L6 antigen (GI 476343). Northern analysis shows that the expression of TUAN is associated with cell proliferation and inflammation.

Therefore, in one embodiment, TUAN or a fragment or derivative thereof may be added  
10 to cells to stimulate cell proliferation. In particular, TUAN may be added to a cell or cells *in vivo* using delivery mechanisms such as liposomes, viral based vectors, or electroinjection for the purpose of promoting regeneration or cell differentiation of the cell or cells. Similarly, TUAN may be added to a cell, cell line, tissue or organ culture *in vitro* or *ex vivo* to stimulate cell proliferation for use in heterologous or autologous transplantation. In some cases, the cell will  
15 have been selected for its ability to fight an infection or a cancer or to correct a genetic defect in a disease such as sickle cell anemia,  $\beta$  thalassemia, cystic fibrosis, or Huntington's chorea.

In another embodiment, an agonist which is specific for TUAN may be administered to a cell to stimulate cell proliferation, as described above.

In still another embodiment, a vector capable of expressing TUAN, or a fragment or a  
20 derivative thereof, may be administered to a cell to stimulate cell proliferation, as described above.

In another embodiment, an antagonist or an inhibitor of TUAN, or a fragment or a derivative thereof, may be administered to a subject to prevent or treat a disorder associated with cell proliferation. Disorders of cell proliferation include various types of cancer including, but  
25 not limited to, adenocarcinoma, leukemia, lymphoma, melanoma, myeloma, sarcoma, and teratocarcinoma, and particularly, cancers of the adrenal gland, bladder, bone, bone marrow, brain, breast, cervix, gall bladder, ganglia, gastrointestinal tract, heart, kidney, liver, lung, muscle, ovary, pancreas, parathyroid, penis, prostate, salivary glands, skin, spleen, testis, thymus, thyroid, and uterus. In one aspect, an antibody specific for TUAN may be used directly as an antagonist,  
30 or indirectly as a targeting or delivery mechanism for bringing a pharmaceutical agent to cells or tissue which express TUAN.

In still another embodiment, a vector expressing the complementary sequence or antisense of the polynucleotide encoding TUAN, or a fragment or a derivative thereof, may be administered

to a subject to prevent or treat a disorder associated with cell proliferation including, but not limited to, those listed above.

In a further embodiment, an antagonist or an inhibitor of TUAN or a fragment or a derivative thereof, may be administered to a subject to prevent or treat inflammation of any type and, in particular, that which results from a particular disorder. Such disorders with associated inflammation include, but are not limited to, Addison's disease, adult respiratory distress syndrome, allergies, anemia, asthma, atherosclerosis, bronchitis, cholecystitis, Crohn's disease, ulcerative colitis, atopic dermatitis, dermatomyositis, diabetes mellitus, emphysema, atrophic gastritis, glomerulonephritis, gout, Graves' disease, hypereosinophilia, irritable bowel syndrome, lupus erythematosus, multiple sclerosis, myasthenia gravis, myocardial or pericardial inflammation, osteoarthritis, osteoporosis, pancreatitis, polymyositis, rheumatoid arthritis, scleroderma, Sjögren's syndrome, and autoimmune thyroiditis; complications of cancer, hemodialysis, extracorporeal circulation; viral, bacterial, fungal, parasitic, protozoal, and helminthic infections and trauma. In one aspect, an antibody specific for TUAN may be used directly as an antagonist, or indirectly as a targeting or delivery mechanism for bringing a pharmaceutical agent to cells or tissue which express TUAN.

In another further embodiment, a vector expressing the complementary sequence or antisense of the polynucleotide encoding TUAN, or a fragment or a derivative thereof, may be administered to a subject to prevent or treat inflammation of any type including, but not limited to, those listed above.

In other embodiments, any of the therapeutic proteins, antagonists, antibodies, agonists, complementary or antisense sequences or vectors of the invention may be administered in combination with other appropriate therapeutic agents. Selection of the appropriate agents for use in combination therapy may be made by one of ordinary skill in the art, according to conventional pharmaceutical principles. The combination of therapeutic agents may act synergistically to effect the treatment or prevention of the various disorders described above. Using this approach, one may be able to achieve therapeutic efficacy with lower dosages of each agent, thus reducing the potential for adverse side effects.

Antagonists or inhibitors of TUAN may be produced using methods which are generally known in the art. In particular, purified TUAN may be used to produce antibodies or to screen libraries of pharmaceutical agents to identify those which specifically bind TUAN.

Antibodies to TUAN may be generated using methods that are well known in the art. Such antibodies may include, but are not limited to, polyclonal, monoclonal, chimeric, single



chain, Fab fragments, and fragments produced by a Fab expression library. Neutralizing antibodies, (i.e., those which inhibit dimer formation) are especially preferred for therapeutic use.

For the production of antibodies, various hosts including goats, rabbits, rats, mice, humans, and others, may be immunized by injection with TUAN or any fragment or oligopeptide thereof which has immunogenic properties. Depending on the host species, various adjuvants may be used to increase immunological response. Such adjuvants include, but are not limited to, Freund's, mineral gels such as aluminum hydroxide, and surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, and dinitrophenol. Among adjuvants used in humans, BCG (bacilli Calmette-Guerin) and Corynebacterium parvum are especially preferable.

It is preferred that the peptides, fragments, or oligopeptides used to induce antibodies to TUAN have an amino acid sequence consisting of at least five amino acids, and more preferably at least 10 amino acids. It is also preferable that they are identical to a portion of the amino acid sequence of the natural protein, and they may contain the entire amino acid sequence of a small, naturally occurring molecule. Short stretches of TUAN amino acids may be fused with those of another protein such as keyhole limpet hemocyanin and antibody produced against the chimeric molecule.

Monoclonal antibodies to TUAN may be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include, but are not limited to, the hybridoma technique, the human B-cell hybridoma technique, and the EBV-hybridoma technique (Kohler, G. et al. (1975) Nature 256:495-497; Kozbor, D. et al. (1985) J. Immunol. Methods 81:31-42; Cote, R.J. et al. (1983) Proc. Natl. Acad. Sci. 80:2026-2030; Cole, S.P. et al. (1984) Mol. Cell Biol. 62:109-120).

In addition, techniques developed for the production of "chimeric antibodies", the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity can be used (Morrison, S.L. et al. (1984) Proc. Natl. Acad. Sci. 81:6851-6855; Neuberger, M.S. et al. (1984) Nature 312:604-608; Takeda, S. et al. (1985) Nature 314:452-454). Alternatively, techniques described for the production of single chain antibodies may be adapted, using methods known in the art, to produce TUAN-specific single chain antibodies. Antibodies with related specificity, but of distinct idiotypic composition, may be generated by chain shuffling from random combinatorial immunoglobulin libraries (Burton D.R. (1991) Proc. Natl. Acad. Sci. 88:11120-3).

Antibodies specific for TUAN may also be produced by inducing in vivo production in

the lymphocyte population or by screening recombinant immunoglobulin libraries or panels of highly specific binding reagents as disclosed in the literature (Orlandi, R. et al. (1989) Proc. Natl. Acad. Sci. 86: 3833-3837; Winter, G. et al. (1991) Nature 349:293-299).

Antibody fragments which contain specific binding sites for TUAN may also be  
5 generated. For example, such fragments include, but are not limited to, the F(ab')<sub>2</sub> fragments which can be produced by pepsin digestion of the antibody molecule and the Fab fragments which can be generated by reducing the disulfide bridges of the F(ab')<sub>2</sub> fragments. Alternatively, Fab expression libraries may be constructed to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity (Huse, W.D. et al. (1989) Science 254:1275-1281).

10 Various immunoassays may be used for screening to identify antibodies having the desired specificity. Numerous protocols for competitive binding or immunoradiometric assays using either polyclonal or monoclonal antibodies with established specificities are well known in the art. Such immunoassays typically involve the measurement of complex formation between TUAN and its specific antibody. A two-site, monoclonal-based immunoassay utilizing  
15 monoclonal antibodies reactive to two non-interfering TUAN epitopes is preferred, but a competitive binding assay may also be employed (Maddox, supra).

In another embodiment of the invention, the polynucleotides encoding TUAN, or any fragment thereof, or antisense molecules, may be used for therapeutic purposes. In one aspect, antisense to the polynucleotide encoding TUAN may be used in situations in which it would be  
20 desirable to block the transcription of the mRNA. In particular, cells may be transformed with sequences complementary to polynucleotides encoding TUAN. Thus, antisense molecules may be used to modulate TUAN activity, or to achieve regulation of gene function. Such technology is now well known in the art, and sense or antisense oligomers or larger fragments, can be designed from various locations along the coding or control regions of sequences encoding  
25 TUAN.

Expression vectors derived from retro viruses, adenovirus, herpes or vaccinia viruses, or from various bacterial plasmids may be used for delivery of nucleotide sequences to the targeted organ, tissue or cell population. Methods which are well known to those skilled in the art can be used to construct recombinant vectors which will express antisense molecules complementary to  
30 the polynucleotides of the gene encoding TUAN. These techniques are described both in Sambrook et al. (supra) and in Ausubel et al. (supra).

Genes encoding TUAN can be turned off by transforming a cell or tissue with expression vectors which express high levels of a polynucleotide or fragment thereof which encodes TUAN.

Such constructs may be used to introduce untranslatable sense or antisense sequences into a cell. Even in the absence of integration into the DNA, such vectors may continue to transcribe RNA molecules until they are disabled by endogenous nucleases. Transient expression may last for a month or more with a non-replicating vector and even longer if appropriate replication elements  
5 are part of the vector system.

As mentioned above, modifications of gene expression can be obtained by designing antisense molecules, DNA, RNA, or PNA, to the control regions of the gene encoding TUAN, i.e., the promoters, enhancers, and introns. Oligonucleotides derived from the transcription initiation site, e.g., between positions -10 and +10 from the start site, are preferred. Similarly,  
10 inhibition can be achieved using "triple helix" base-pairing methodology. Triple helix pairing is useful because it causes inhibition of the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors, or regulatory molecules. Recent therapeutic advances using triplex DNA have been described in the literature (Gee, J.E. et al. (1994) In: Huber, B.E. and B.I. Carr, Molecular and Immunologic Approaches, Futura Publishing Co., Mt.  
15 Kisco, NY). The antisense molecules may also be designed to block translation of mRNA by preventing the transcript from binding to ribosomes.

Ribozymes, enzymatic RNA molecules, may also be used to catalyze the specific cleavage of RNA. The mechanism of ribozyme action involves sequence-specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage.  
20 Examples which may be used include engineered hammerhead motif ribozyme molecules that can specifically and efficiently catalyze endonucleolytic cleavage of sequences encoding TUAN.

Specific ribozyme cleavage sites within any potential RNA target are initially identified by scanning the target molecule for ribozyme cleavage sites which include the following sequences: GUA, GUU, and GUC. Once identified, short RNA sequences of between 15 and 20  
25 ribonucleotides corresponding to the region of the target gene containing the cleavage site may be evaluated for secondary structural features which may render the oligonucleotide inoperable. The suitability of candidate targets may also be evaluated by testing accessibility to hybridization with complementary oligonucleotides using ribonuclease protection assays.

Antisense molecules and ribozymes of the invention may be prepared by any method  
30 known in the art for the synthesis of nucleic acid molecules. These include techniques for chemically synthesizing oligonucleotides such as solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by *in vitro* and *in vivo* transcription of DNA sequences encoding TUAN. Such DNA sequences may be incorporated into a wide

variety of vectors with suitable RNA polymerase promoters such as T7 or SP6. Alternatively, these cDNA constructs that synthesize antisense RNA constitutively or inducibly can be introduced into cell lines, cells, or tissues.

RNA molecules may be modified to increase intracellular stability and half-life. Possible  
5 modifications include, but are not limited to, the addition of flanking sequences at the 5' and/or 3' ends of the molecule or the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages within the backbone of the molecule. This concept is inherent in the production of PNAs and can be extended in all of these molecules by the inclusion of nontraditional bases such as inosine, queosine, and wybutosine, as well as acetyl-, methyl-, thio-, and similarly modified  
10 forms of adenine, cytidine, guanine, thymine, and uridine which are not as easily recognized by endogenous endonucleases.

Many methods for introducing vectors into cells or tissues are available and equally suitable for use in vivo, in vitro, and ex vivo. For ex vivo therapy, vectors may be introduced into stem cells taken from the patient and clonally propagated for autologous transplant back into  
15 that same patient. Delivery by transfection and by liposome injections may be achieved using methods which are well known in the art.

Any of the therapeutic methods described above may be applied to any subject in need of such therapy, including, for example, mammals such as dogs, cats, cows, horses, rabbits, monkeys, and most preferably, humans.

20 An additional embodiment of the invention relates to the administration of a pharmaceutical composition, in conjunction with a pharmaceutically acceptable carrier, for any of the therapeutic effects discussed above. Such pharmaceutical compositions may consist of TUAN, antibodies to TUAN, mimetics, agonists, antagonists, or inhibitors of TUAN. The compositions may be administered alone or in combination with at least one other agent, such as  
25 stabilizing compound, which may be administered in any sterile, biocompatible pharmaceutical carrier, including, but not limited to, saline, buffered saline, dextrose, and water. The compositions may be administered to a patient alone, or in combination with other agents, drugs or hormones.

The pharmaceutical compositions utilized in this invention may be administered by any  
30 number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual, or rectal means.

In addition to the active ingredients, these pharmaceutical compositions may contain

suitable pharmaceutically-acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Further details on techniques for formulation and administration may be found in the latest edition of Remington's Pharmaceutical Sciences (Maack Publishing Co., Easton,

5 PA).

Pharmaceutical compositions for oral administration can be formulated using pharmaceutically acceptable carriers well known in the art in dosages suitable for oral administration. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for ingestion by  
10 the patient.

Pharmaceutical preparations for oral use can be obtained through combination of active compounds with solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are carbohydrate or protein fillers, such as sugars, including lactose, sucrose,  
15 mannitol, or sorbitol; starch from corn, wheat, rice, potato, or other plants; cellulose, such as methyl cellulose, hydroxypropylmethyl-cellulose, or sodium carboxymethylcellulose; gums including arabic and tragacanth; and proteins such as gelatin and collagen. If desired, disintegrating or solubilizing agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, alginic acid, or a salt thereof, such as sodium alginate.

20 Dragee cores may be used in conjunction with suitable coatings, such as concentrated sugar solutions, which may also contain gum arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for product identification or to characterize the quantity of active compound, i.e., dosage.

25 Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a coating, such as glycerol or sorbitol. Push-fit capsules can contain active ingredients mixed with a filler or binders, such as lactose or starches, lubricants, such as talc or magnesium stearate, and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty  
30 oils, liquid, or liquid polyethylene glycol with or without stabilizers.

Pharmaceutical formulations suitable for parenteral administration may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks's solution, Ringer's solution, or physiologically buffered saline. Aqueous injection suspensions may contain

substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

For topical or nasal administration, penetrants appropriate to the particular barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

10 The pharmaceutical compositions of the present invention may be manufactured in a manner that is known in the art, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping, or lyophilizing processes.

The pharmaceutical composition may be provided as a salt and can be formed with many acids, including but not limited to, hydrochloric, sulfuric, acetic, lactic, tartaric, malic, succinic, etc. Salts tend to be more soluble in aqueous or other protonic solvents than are the corresponding free base forms. In other cases, the preferred preparation may be a lyophilized powder which may contain any or all of the following: 1-50 mM histidine, 0.1%-2% sucrose, and 2-7% mannitol, at a pH range of 4.5 to 5.5, that is combined with buffer prior to use.

After pharmaceutical compositions have been prepared, they can be placed in an appropriate container and labeled for treatment of an indicated condition. For administration of TUAN, such labeling would include amount, frequency, and method of administration.

Pharmaceutical compositions suitable for use in the invention include compositions wherein the active ingredients are contained in an effective amount to achieve the intended purpose. The determination of an effective dose is well within the capability of those skilled in the art.

For any compound, the therapeutically effective dose can be estimated initially either in cell culture assays, e.g., of neoplastic cells, or in animal models, usually mice, rabbits, dogs, or pigs. The animal model may also be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

A therapeutically effective dose refers to that amount of active ingredient, for example TUAN or fragments thereof, antibodies of TUAN, agonists, antagonists or inhibitors of TUAN, which ameliorates the symptoms or condition. Therapeutic efficacy and toxicity may be

determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., ED50 (the dose therapeutically effective in 50% of the population) and LD50 (the dose lethal to 50% of the population). The dose ratio between therapeutic and toxic effects is the therapeutic index, and it can be expressed as the ratio, LD50/ED50. Pharmaceutical compositions which  
5 exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies is used in formulating a range of dosage for human use. The dosage contained in such compositions is preferably within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage varies within this range depending upon the dosage form employed, sensitivity of the patient, and the route of administration.

10 The exact dosage will be determined by the practitioner, in light of factors related to the subject that requires treatment. Dosage and administration are adjusted to provide sufficient levels of the active moiety or to maintain the desired effect. Factors which may be taken into account include the severity of the disease state, general health of the subject, age, weight, and gender of the subject, diet, time and frequency of administration, drug combination(s), reaction  
15 sensitivities, and tolerance/response to therapy. Long-acting pharmaceutical compositions may be administered every 3 to 4 days, every week, or once every two weeks depending on half-life and clearance rate of the particular formulation.

Normal dosage amounts may vary from 0.1 to 100,000 micrograms, up to a total dose of about 1 g, depending upon the route of administration. Guidance as to particular dosages and  
20 methods of delivery is provided in the literature and generally available to practitioners in the art. Those skilled in the art will employ different formulations for nucleotides than for proteins or their inhibitors. Similarly, delivery of polynucleotides or polypeptides will be specific to particular cells, conditions, locations, etc.

#### DIAGNOSTICS

25 In another embodiment, antibodies which specifically bind TUAN may be used for the diagnosis of conditions or diseases characterized by expression of TUAN, or in assays to monitor patients being treated with TUAN, agonists, antagonists or inhibitors. The antibodies useful for diagnostic purposes may be prepared in the same manner as those described above for therapeutics. Diagnostic assays for TUAN include methods which utilize the antibody and a  
30 label to detect TUAN in human body fluids or extracts of cells or tissues. The antibodies may be used with or without modification, and may be labeled by joining them, either covalently or non-covalently, with a reporter molecule. A wide variety of reporter molecules which are known in the art may be used, several of which are described above.

A variety of protocols including ELISA, RIA, and FACS for measuring TUAN are known in the art and provide a basis for diagnosing altered or abnormal levels of TUAN expression. Normal or standard values for TUAN expression are established by combining body fluids or cell extracts taken from normal mammalian subjects, preferably human, with antibody to TUAN  
5 under conditions suitable for complex formation. The amount of standard complex formation may be quantified by various methods, but preferably by photometric means. Quantities of TUAN expressed in subject, control and disease, samples from biopsied tissues are compared with the standard values. Deviation between standard and subject values establishes the parameters for diagnosing disease.

10 In another embodiment of the invention, the polynucleotides encoding TUAN may be used for diagnostic purposes. The polynucleotides which may be used include oligonucleotide sequences, antisense RNA and DNA molecules, and PNAs. The polynucleotides may be used to detect and quantitate gene expression in biopsied tissues in which expression of TUAN may be correlated with disease. The diagnostic assay may be used to distinguish between absence,  
15 presence, and excess expression of TUAN, and to monitor regulation of TUAN levels during therapeutic intervention.

In one aspect, hybridization with PCR probes which are capable of detecting polynucleotide sequences, including genomic sequences, encoding TUAN or closely related molecules, may be used to identify nucleic acid sequences which encode TUAN. The specificity  
20 of the probe, whether it is made from a highly specific region, e.g., 10 unique nucleotides in the 5' regulatory region, or a less specific region, e.g., especially in the 3' coding region, and the stringency of the hybridization or amplification (maximal, high, intermediate, or low) will determine whether the probe identifies only naturally occurring sequences encoding TUAN, alleles, or related sequences.

25 Probes may also be used for the detection of related sequences, and should preferably contain at least 50% of the nucleotides from any of the TUAN encoding sequences. The hybridization probes of the subject invention may be DNA or RNA and derived from the nucleotide sequence of SEQ ID NO:2 or from genomic sequence including promoter, enhancer elements, and introns of the naturally occurring TUAN.

30 Means for producing specific hybridization probes for DNAs encoding TUAN include the cloning of nucleic acid sequences encoding TUAN or TUAN derivatives into vectors for the production of mRNA probes. Such vectors are known in the art, commercially available, and may be used to synthesize RNA probes *in vitro* by means of the addition of the appropriate RNA



polymerases and the appropriate labeled nucleotides. Hybridization probes may be labeled by a variety of reporter groups, for example, radionuclides such as  $^{32}\text{P}$  or  $^{35}\text{S}$ , or enzymatic labels, such as alkaline phosphatase coupled to the probe via avidin/biotin coupling systems, and the like.

5 Polynucleotide sequences encoding TUAN may be used for the diagnosis of disorders associated with the expression of TUAN. Examples of such disorders include: various types of cancer such as adenocarcinoma, leukemia, lymphoma, melanoma, myeloma, sarcoma, and teratocarcinoma, and particularly, cancers of the adrenal gland, bladder, bone, bone marrow, brain, breast, cervix, gall bladder, ganglia, gastrointestinal tract, heart, kidney, liver, lung, muscle,  
10 ovary, pancreas, parathyroid, penis, prostate, salivary glands, skin, spleen, testis, thymus, thyroid, and uterus; disorders associated with inflammation such as Addison's disease, adult respiratory distress syndrome, allergies, anemia, asthma, atherosclerosis, bronchitis, cholecystitis, Crohn's disease, ulcerative colitis, atopic dermatitis, dermatomyositis, diabetes mellitus, emphysema, atrophic gastritis, glomerulonephritis, gout, Graves' disease, hypereosinophilia, irritable bowel  
15 syndrome, lupus erythematosus, multiple sclerosis, myasthenia gravis, myocardial or pericardial inflammation, osteoarthritis, osteoporosis, pancreatitis, polymyositis, rheumatoid arthritis, scleroderma, Sjögren's syndrome, and autoimmune thyroiditis; complications of cancer, hemodialysis, extracorporeal circulation; viral, bacterial, fungal, parasitic, protozoal, and helminthic infections and trauma. The polynucleotide sequences encoding TUAN may be used in  
20 Southern or northern analysis, dot blot, or other membrane-based technologies; in PCR technologies; or in dip stick, pin, ELISA or chip assays utilizing fluids or tissues from patient biopsies to detect altered TUAN expression. Such qualitative or quantitative methods are well known in the art.

In a particular aspect, the nucleotide sequences encoding TUAN may be useful in assays  
25 that detect activation or induction of various cancers, particularly those mentioned above. The nucleotide sequences encoding TUAN may be labeled by standard methods, and added to a fluid or tissue sample from a patient under conditions suitable for the formation of hybridization complexes. After a suitable incubation period, the sample is washed and the signal is quantitated and compared with a standard value. If the amount of signal in the biopsied or extracted sample  
30 is significantly altered from that of a comparable control sample, the nucleotide sequences have hybridized with nucleotide sequences in the sample, and the presence of altered levels of nucleotide sequences encoding TUAN in the sample indicates the presence of the associated disease. Such assays may also be used to evaluate the efficacy of a particular therapeutic

treatment regimen in animal studies, in clinical trials, or in monitoring the treatment of an individual patient.

In order to provide a basis for the diagnosis of disease associated with expression of TUAN, a normal or standard profile for expression is established. This may be accomplished by  
5 combining body fluids or cell extracts taken from normal subjects, either animal or human, with a sequence, or a fragment thereof, which encodes TUAN, under conditions suitable for hybridization or amplification. Standard hybridization may be quantified by comparing the values obtained from normal subjects with those from an experiment where a known amount of a substantially purified polynucleotide is used. Standard values obtained from normal samples may  
10 be compared with values obtained from samples from patients who are symptomatic for disease. Deviation between standard and subject values is used to establish the presence of disease.

Once disease is established and a treatment protocol is initiated, hybridization assays may be repeated on a regular basis to evaluate whether the level of expression in the patient begins to approximate that which is observed in the normal patient. The results obtained from successive  
15 assays may be used to show the efficacy of treatment over a period ranging from several days to months.

With respect to cancer, the presence of a relatively high amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical  
20 symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

Additional diagnostic uses for oligonucleotides designed from the sequences encoding TUAN may involve the use of PCR. Such oligomers may be chemically synthesized, generated  
25 enzymatically, or produced from a recombinant source. Oligomers will preferably consist of two nucleotide sequences, one with sense orientation (5'→3') and another with antisense (3'←5'), employed under optimized conditions for identification of a specific gene or condition. The same two oligomers, nested sets of oligomers, or even a degenerate pool of oligomers may be employed under less stringent conditions for detection and/or quantitation of closely related DNA  
30 or RNA sequences.

Methods which may also be used to quantitate the expression of TUAN include radiolabeling or biotinylating nucleotides, coamplification of a control nucleic acid, and standard curves onto which the experimental results are interpolated (Melby, P.C. et al. (1993) J.

Immunol. Methods, 159:235-244; Duplaa, C. et al. (1993) Anal. Biochem. 229-236). The speed of quantitation of multiple samples may be accelerated by running the assay in an ELISA format where the oligomer of interest is presented in various dilutions and a spectrophotometric or colorimetric response gives rapid quantitation.

5 In another embodiment of the invention, the nucleic acid sequences which encode TUAN may also be used to generate hybridization probes which are useful for mapping the naturally occurring genomic sequence. The sequences may be mapped to a particular chromosome or to a specific region of the chromosome using well known techniques. Such techniques include FISH, FACS, or artificial chromosome constructions, such as yeast artificial chromosomes, bacterial  
10 artificial chromosomes, bacterial P1 constructions or single chromosome cDNA libraries as reviewed in Price, C.M. (1993) Blood Rev. 7:127-134, and Trask, B.J. (1991) Trends Genet. 7:149-154.

FISH (as described in Verma et al. (1988) Human Chromosomes: A Manual of Basic Techniques, Pergamon Press, New York, NY) may be correlated with other physical chromosome  
15 mapping techniques and genetic map data. Examples of genetic map data can be found in the 1994 Genome Issue of Science (265:1981f). Correlation between the location of the gene encoding TUAN on a physical chromosomal map and a specific disease, or predisposition to a specific disease, may help delimit the region of DNA associated with that genetic disease. The nucleotide sequences of the subject invention may be used to detect differences in gene sequences  
20 between normal, carrier, or affected individuals.

In situ hybridization of chromosomal preparations and physical mapping techniques such as linkage analysis using established chromosomal markers may be used for extending genetic maps. Often the placement of a gene on the chromosome of another mammalian species, such as mouse, may reveal associated markers even if the number or arm of a particular human  
25 chromosome is not known. New sequences can be assigned to chromosomal arms, or parts thereof, by physical mapping. This provides valuable information to investigators searching for disease genes using positional cloning or other gene discovery techniques. Once the disease or syndrome has been crudely localized by genetic linkage to a particular genomic region, for example, AT to 11q22-23 (Gatti, R.A. et al. (1988) Nature 336:577-580), any sequences mapping  
30 to that area may represent associated or regulatory genes for further investigation. The nucleotide sequence of the subject invention may also be used to detect differences in the chromosomal location due to translocation, inversion, etc. among normal, carrier, or affected individuals.

In another embodiment of the invention, TUAN, its catalytic or immunogenic fragments

or oligopeptides thereof, can be used for screening libraries of compounds in any of a variety of drug screening techniques. The fragment employed in such screening may be free in solution, affixed to a solid support, borne on a cell surface, or located intracellularly. The formation of binding complexes, between TUAN and the agent being tested, may be measured.

- 5 Another technique for drug screening which may be used provides for high throughput screening of compounds having suitable binding affinity to the protein of interest as described in published PCT application WO84/03564. In this method, as applied to TUAN large numbers of different small test compounds are synthesized on a solid substrate, such as plastic pins or some other surface. The test compounds are reacted with TUAN, or fragments thereof, and washed.
- 10 Bound TUAN is then detected by methods well known in the art. Purified TUAN can also be coated directly onto plates for use in the aforementioned drug screening techniques. Alternatively, non-neutralizing antibodies can be used to capture the peptide and immobilize it on a solid support.

- In another embodiment, one may use competitive drug screening assays in which
- 15 neutralizing antibodies capable of binding TUAN specifically compete with a test compound for binding TUAN. In this manner, the antibodies can be used to detect the presence of any peptide which shares one or more antigenic determinants with TUAN.

- In additional embodiments, the nucleotide sequences which encode TUAN may be used in any molecular biology techniques that have yet to be developed, provided the new techniques
- 20 rely on properties of nucleotide sequences that are currently known, including, but not limited to, such properties as the triplet genetic code and specific base pair interactions.

The examples below are provided to illustrate the subject invention and are not included for the purpose of limiting the invention.

#### INDUSTRIAL APPLICABILITY

##### 25 I COLNNOT19 cDNA Library Construction

- The COLNNOT19 library was constructed using 1 microgram of polyA RNA isolated from the unaffected cecal tissue of an 18-year-old Caucasian female with irritable bowel syndrome (IBS). The cecal tissue, along with the appendix and ileum tissue, were removed during bowel anastomosis. Pathology indicated Crohn's disease, involving 15 cm of the small
- 30 bowel. The cecum and appendix were unremarkable, and the margins were uninvolved. The patient presented with abdominal pain and regional enteritis. Patient history included osteoporosis of the vertebra and abnormal blood chemistry.

The frozen tissue was immediately homogenized and lysed using a Brinkmann

Homogenizer Polytron-PT 3000 (Brinkmann Instruments, Inc., Westbury, NY) in guanidinium isothiocyanate solution. Lysates were then loaded on a 5.7 M CsCl cushion and ultracentrifuged in a SW28 swinging bucket rotor for 18 hours at 25,000 rpm at ambient temperature. The RNA was extracted once with acid phenol at pH 4.0 and once with phenol chloroform at pH 8.0 and  
5 precipitated using 0.3 M sodium acetate and 2.5 volumes of ethanol, resuspended in DEPC-treated water and DNase treated for 25 min at 37°C. The reaction was stopped with an equal volume of acid phenol, and the RNA was isolated using the Qiagen Oligotex kit (QIAGEN Inc, Chatsworth, CA) and used to construct the cDNA library.

The mRNA was handled according to the recommended protocols in the SuperScript  
10 Plasmid System for cDNA Synthesis and Plasmid Cloning (Cat. #18248-013; Gibco/BRL, Gaithersburg, MD). cDNAs were fractionated on a Sepharose CL4B column (Cat. #275105, Pharmacia), and those cDNAs exceeding 400 bp were ligated into an Incyte vector, pINCY 1. The plasmid pINCY I was subsequently transformed into DH5a™ competent cells (Cat. #18258-012, Gibco/BRL).

## 15 II Isolation and Sequencing of cDNA Clones

Plasmid DNA was released from the cells and purified using the REAL Prep 96 plasmid Kit (Cat. # 26173; Qiagen). This kit enabled the simultaneous purification of 96 samples in a 96-well block using multi-channel reagent dispensers. The recommended protocol was employed except for the following changes: 1) the 96 wells were each filled with only 1 ml of sterile  
20 Terrific Broth (Cat. # 22711, Gibco/BRL) with carbenicillin at 25 mg/L and glycerol at 0.4%; 2) the bacteria were cultured for 19 hours after the wells were inoculated and then lysed with 300 µl of lysis buffer; and 3) following isopropanol precipitation, the plasmid DNA pellet was resuspended in 0.1 ml of distilled water. After the last step in the protocol, samples were transferred to a Beckman 96-well block for storage at 4°C.

25 The cDNAs were sequenced by the method of Sanger F and AR Coulson (1975; J Mol Biol 94:441f), using a Hamilton Micro Lab 2200 (Hamilton, Reno, NV) in combination with four Peltier Thermal Cyclers (PTC200; MJ Research, Watertown, MA) and Applied Biosystems 377 DNA Sequencing Systems (Perkin Elmer), and reading frame was determined.

## III Homology Searching of cDNA Clones and Their Deduced Proteins

30 After the reading frame was determined, the nucleotide sequences of the Sequence Listing or amino acid sequences deduced from them were used as query sequences against databases such as GenBank, SwissProt, BLOCKS, and Pima II. These databases which contain previously identified and annotated sequences, were searched for regions of homology (similarity) using

BLAST, which stands for Basic Local Alignment Search Tool (Altschul (1993) *supra*, Altschul (1990) *supra*).

BLAST produced alignments of both nucleotide and amino acid sequences to determine sequence similarity. Because of the local nature of the alignments, BLAST was especially useful in determining exact matches or in identifying homologs which may be of prokaryotic (bacterial) or eukaryotic (animal, fungal, or plant) origin. Other algorithms such as the one described in Smith et al. (1992, *Protein Engineering* 5:35-51), incorporated herein by reference, could have been used when dealing with primary sequence patterns and secondary structure gap penalties. The sequences disclosed in this application have lengths of at least 49 nucleotides, and no more than 12% uncalled bases (where N is recorded rather than A, C, G, or T).

The BLAST approach, as detailed in Karlin et al. (*supra*) and incorporated herein by reference, searched for matches between a query sequence and a database sequence. BLAST evaluated the statistical significance of any matches found, and reported only those matches that satisfy the user-selected threshold of significance. In this application, threshold was set at  $10^{-25}$  for nucleotides and  $10^{-14}$  for peptides.

#### IV Northern Analysis

Northern analysis is a laboratory technique used to detect the presence of a transcript of a gene and involves the hybridization of a labeled nucleotide sequence to a membrane on which RNAs from a particular cell type or tissue have been bound (Sambrook et al., *supra*).

Analogous computer techniques using BLAST (Altschul, S.F. 1993 and 1990, *supra*) are used to search for identical or related molecules in nucleotide databases such as GenBank or the LIFESEQ™ database (Incyte Pharmaceuticals). This analysis is much faster than multiple, membrane-based hybridizations. In addition, the sensitivity of the computer search can be modified to determine whether any particular match is categorized as exact or homologous.

The basis of the search is the product score which is defined as:

$$\frac{\% \text{ sequence identity} \times \% \text{ maximum BLAST score}}{100}$$

The product score takes into account both the degree of similarity between two sequences and the length of the sequence match. For example, with a product score of 40, the match will be exact within a 1-2% error; and at 70, the match will be exact. Homologous molecules are usually identified by selecting those which show product scores between 15 and 40, although lower scores may identify related molecules.

The results of northern analysis are reported as a list of libraries in which the transcript

encoding TUAN occurs. Abundance and percent abundance are also reported. Abundance directly reflects the number of times a particular transcript is represented in a cDNA library, and percent abundance is abundance divided by the total number of sequences examined in the cDNA library.

#### 5 V Extension of TUAN-Encoding Polynucleotides

Nucleic acid sequence of Incyte clone 1634851 or SEQ ID NO:2 is used to design oligonucleotide primers for extending a partial nucleotide sequence to full length or for obtaining 5' or 3', intron or other control sequences from genomic libraries. One primer is synthesized to initiate extension in the antisense direction (XLR) and the other is synthesized to extend sequence  
 10 in the sense direction (XLF). Primers are used to facilitate the extension of the known sequence "outward" generating amplicons containing new, unknown nucleotide sequence for the region of interest. The initial primers are designed from the cDNA using OLIGO 4.06 (National Biosciences), or another appropriate program, to be 22-30 nucleotides in length, to have a GC content of 50% or more, and to anneal to the target sequence at temperatures about 68°-72° C.  
 15 Any stretch of nucleotides which would result in hairpin structures and primer-primer dimerizations is avoided.

The original, selected cDNA libraries, or a human genomic library are used to extend the sequence; the latter is most useful to obtain 5' upstream regions. If more extension is necessary or desired, additional sets of primers are designed to further extend the known region.

20 By following the instructions for the XL-PCR kit (Perkin Elmer) and thoroughly mixing the enzyme and reaction mix, high fidelity amplification is obtained. Beginning with 40 pmol of each primer and the recommended concentrations of all other components of the kit, PCR is performed using the Peltier Thermal Cycler (PTC200; M.J. Research, Watertown, MA) and the following parameters:

25	Step 1	94° C for 1 min (initial denaturation)
	Step 2	65° C for 1 min
	Step 3	68° C for 6 min
	Step 4	94° C for 15 sec
	Step 5	65° C for 1 min
30	Step 6	68° C for 7 min
	Step 7	Repeat step 4-6 for 15 additional cycles
	Step 8	94° C for 15 sec
	Step 9	65° C for 1 min
	Step 10	68° C for 7:15 min
35	Step 11	Repeat step 8-10 for 12 cycles
	Step 12	72° C for 8 min
	Step 13	4° C (and holding)

A 5-10  $\mu$ l aliquot of the reaction mixture is analyzed by electrophoresis on a low concentration (about 0.6-0.8%) agarose mini-gel to determine which reactions were successful in extending the sequence. Bands thought to contain the largest products are selected and removed from the gel. Further purification involves using a commercial gel extraction method such as QIAQuick™ (QIAGEN Inc., Chatsworth, CA). After recovery of the DNA, Klenow enzyme is used to trim single-stranded, nucleotide overhangs creating blunt ends which facilitate religation and cloning.

After ethanol precipitation, the products are redissolved in 13  $\mu$ l of ligation buffer, 1  $\mu$ l T4-DNA ligase (15 units) and 1  $\mu$ l T4 polynucleotide kinase are added, and the mixture is incubated at room temperature for 2-3 hours or overnight at 16° C. Competent *E. coli* cells (in 40  $\mu$ l of appropriate media) are transformed with 3  $\mu$ l of ligation mixture and cultured in 80  $\mu$ l of SOC medium (Sambrook et al., supra). After incubation for one hour at 37° C, the whole transformation mixture is plated on Luria Bertani (LB)-agar (Sambrook et al., supra) containing 2x Carb. The following day, several colonies are randomly picked from each plate and cultured in 150  $\mu$ l of liquid LB/2x Carb medium placed in an individual well of an appropriate, commercially-available, sterile 96-well microtiter plate. The following day, 5  $\mu$ l of each overnight culture is transferred into a non-sterile 96-well plate and after dilution 1:10 with water, 5  $\mu$ l of each sample is transferred into a PCR array.

For PCR amplification, 18  $\mu$ l of concentrated PCR reaction mix (3.3x) containing 4 units of rTth DNA polymerase, a vector primer, and one or both of the gene specific primers used for the extension reaction are added to each well. Amplification is performed using the following conditions:

25	Step 1	94° C for 60 sec
	Step 2	94° C for 20 sec
	Step 3	55° C for 30 sec
	Step 4	72° C for 90 sec
30	Step 5	Repeat steps 2-4 for an additional 29 cycles
	Step 6	72° C for 180 sec
	Step 7	4° C (and holding)

Aliquots of the PCR reactions are run on agarose gels together with molecular weight markers. The sizes of the PCR products are compared to the original partial cDNAs, and appropriate clones are selected, ligated into plasmid, and sequenced.

#### VI Labeling and Use of Hybridization Probes

Hybridization probes derived from SEQ ID NO:2 are employed to screen cDNAs,



genomic DNAs, or mRNAs. Although the labeling of oligonucleotides, consisting of about 20 base-pairs, is specifically described, essentially the same procedure is used with larger cDNA fragments. Oligonucleotides are designed using state-of-the-art software such as OLIGO 4.06 (National Biosciences), labeled by combining 50 pmol of each oligomer and 250  $\mu$ Ci of [ $\gamma$ - $^{32}$ P] adenosine triphosphate (Amersham) and T4 polynucleotide kinase (DuPont NEN<sup>®</sup>, Boston, MA). The labeled oligonucleotides are substantially purified with Sephadex G-25 superfine resin column (Pharmacia & Upjohn). A portion containing 10<sup>7</sup> counts per minute of each of the sense and antisense oligonucleotides is used in a typical membrane based hybridization analysis of human genomic DNA digested with one of the following endonucleases (Ase I, Bgl II, Eco RI, Pst I, Xba I, or Pvu II; DuPont NEN<sup>®</sup>).

The DNA from each digest is fractionated on a 0.7 percent agarose gel and transferred to nylon membranes (Nytran Plus, Schleicher & Schuell, Durham, NH). Hybridization is carried out for 16 hours at 40°C. To remove nonspecific signals, blots are sequentially washed at room temperature under increasingly stringent conditions up to 0.1 x saline sodium citrate and 0.5% sodium dodecyl sulfate. After XOMAT AR<sup>™</sup> film (Kodak, Rochester, NY) is exposed to the blots in a Phosphorimager cassette (Molecular Dynamics, Sunnyvale, CA) for several hours, hybridization patterns are compared visually.

## VII Antisense or Complementary Sequences

Antisense molecules or nucleic acid sequences complementary to the TUAN-encoding sequence, or any part thereof, are used to inhibit *in vivo* or *in vitro* expression of naturally occurring TUAN. Although use of antisense oligonucleotides, comprising about 20 base-pairs, is specifically described, essentially the same procedure is used with larger cDNA fragments. An oligonucleotide based on the coding sequences of TUAN, as shown in Figures 1A and 1B, is used to inhibit expression of naturally occurring TUAN. The complementary oligonucleotide is designed from the most unique 5' sequence as shown in Figures 1A and 1B and used either to inhibit transcription by preventing promoter binding to the upstream nontranslated sequence or translation of an TUAN-encoding transcript by preventing the ribosome from binding. Using an appropriate portion of the signal and 5' sequence of SEQ ID NO:2, an effective antisense oligonucleotide includes any 15-20 nucleotides spanning the region which translates into the signal or 5' coding sequence of the polypeptide as shown in Figures 1A and 1B.

## VIII Expression of TUAN

Expression of TUAN is accomplished by subcloning the cDNAs into appropriate vectors and transforming the vectors into host cells. In this case, the cloning vector, pSport, previously

used for the generation of the cDNA library is used to express TUAN in *E. coli*. Upstream of the cloning site, this vector contains a promoter for  $\beta$ -galactosidase, followed by sequence containing the amino-terminal Met, and the subsequent seven residues of  $\beta$ -galactosidase. Immediately following these eight residues is a bacteriophage promoter useful for transcription and a linker  
5 containing a number of unique restriction sites.

Induction of an isolated, transformed bacterial strain with IPTG using standard methods produces a fusion protein which consists of the first eight residues of  $\beta$ -galactosidase, about 5 to 15 residues of linker, and the full length protein. The signal residues direct the secretion of TUAN into the bacterial growth media which can be used directly in the following assay for  
10 activity.

#### **IX Demonstration of TUAN Activity**

TUAN can be expressed by transforming a mammalian cell line such as COS7, HeLa or CHO with an eukaryotic expression vector encoding TUAN. Eukaryotic expression vectors are commercially available, and the techniques to introduce them into cells are well known to those  
15 skilled in the art. The cells are incubated for 48-72 hours after transformation under conditions appropriate for the cell line to allow expression and accumulation of TUAN.

Extracts containing solubilized proteins are prepared from cells expressing TUAN by methods well known in the art. Portions of the extract containing TUAN are applied to the wells of a polyacrylamide gel and electrophoresed at constant current until a suitable tracking dye, such  
20 as bromophenol blue (Sigma), has migrated to the bottom of the gel. Appropriate control samples, prepared from extracts of untransformed cells and/or cells transformed with vector sequences alone, are electrophoresed in parallel lanes of the gel. Protein standards of known molecular weight (BioRad, Hercules, CA) are run in adjacent lanes to calibrate the gel.

#### **X Production of TUAN Specific Antibodies**

25 TUAN that is substantially purified using PAGE electrophoresis (Sambrook, supra), or other purification techniques, is used to immunize rabbits and to produce antibodies using standard protocols. The amino acid sequence deduced from SEQ ID NO:2 is analyzed using DNASTAR software (DNASTAR Inc) to determine regions of high immunogenicity and a corresponding oligopolypeptide is synthesized and used to raise antibodies by means known to  
30 those of skill in the art. Selection of appropriate epitopes, such as those near the C-terminus or in hydrophilic regions, is described by Ausubel et al. (supra), and others.

Typically, the oligopeptides are 15 residues in length, synthesized using an Applied Biosystems Peptide Synthesizer Model 431A using fmoc-chemistry, and coupled to keyhole

limpet hemocyanin (KLH, Sigma, St. Louis, MO) by reaction with N-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS; Ausubel et al., supra). Rabbits are immunized with the oligopeptide-KLH complex in complete Freund's adjuvant. The resulting antisera are tested for antipeptide activity, for example, by binding the peptide to plastic, blocking with 1% BSA, reacting with rabbit antisera, washing, and reacting with radioiodinated, goat anti-rabbit IgG.

#### **XI Purification of Naturally Occurring TUAN Using Specific Antibodies**

Naturally occurring or recombinant TUAN is substantially purified by immunoaffinity chromatography using antibodies specific for TUAN. An immunoaffinity column is constructed by covalently coupling TUAN antibody to an activated chromatographic resin, such as CnBr-activated Sepharose (Pharmacia & Upjohn). After the coupling, the resin is blocked and washed according to the manufacturer's instructions.

Media containing TUAN is passed over the immunoaffinity column, and the column is washed under conditions that allow the preferential absorbance of TUAN (e.g., high ionic strength buffers in the presence of detergent). The column is eluted under conditions that disrupt antibody/TUAN binding (eg, a buffer of pH 2-3 or a high concentration of a chaotrope, such as urea or thiocyanate ion), and TUAN is collected.

#### **XII Identification of Molecules Which Interact with TUAN**

TUAN or biologically active fragments thereof are labeled with <sup>125</sup>I Bolton-Hunter reagent (Bolton et al. (1973) Biochem. J. 133:529). Candidate molecules previously arrayed in the wells of a multi-well plate are incubated with the labeled TUAN, washed and any wells with labeled TUAN complex are assayed. Data obtained using different concentrations of TUAN are used to calculate values for the number, affinity, and association of TUAN with the candidate molecules.

All publications and patents mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described method and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in molecular biology or related fields are intended to be within the scope of the following claims.

## SEQUENCE LISTING

## (1) GENERAL INFORMATION

- (i) APPLICANT: INCYTE PHARMACEUTICALS, INC.
- (ii) TITLE OF THE INVENTION: TUMOR-ASSOCIATED ANTIGEN
- (iii) NUMBER OF SEQUENCES: 4
- (iv) CORRESPONDENCE ADDRESS:
  - (A) ADDRESSEE: Incyte Pharmaceuticals, Inc.
  - (B) STREET: 3174 Porter Drive
  - (C) CITY: Palo Alto
  - (D) STATE: CA
  - (E) COUNTRY: USA
  - (F) ZIP: 94304
- (v) COMPUTER READABLE FORM:
  - (A) MEDIUM TYPE: Diskette
  - (B) COMPUTER: IBM Compatible
  - (C) OPERATING SYSTEM: DOS
  - (D) SOFTWARE: FastSEQ for Windows Version 2.0
- (vi) CURRENT APPLICATION DATA:
  - (A) PCT APPLICATION NUMBER: To Be Assigned
  - (B) FILING DATE: Herewith
- (vii) PRIOR APPLICATION DATA:
  - (A) APPLICATION NUMBER: US 08/855,261
  - (B) FILING DATE: 13-MAY-1997
- (viii) ATTORNEY/AGENT INFORMATION:
  - (A) NAME: Billings, Lucy J.
  - (B) REGISTRATION NUMBER: 36,749
  - (C) REFERENCE/DOCKET NUMBER: PF-0298 PCT
- (ix) TELECOMMUNICATION INFORMATION:
  - (A) TELEPHONE: 650-855-0555
  - (B) TELEFAX: 650-845-4166

## (2) INFORMATION FOR SEQ ID NO:1:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 197 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (vii) IMMEDIATE SOURCE:

- (A) LIBRARY: COLNNOT19
- (B) CLONE: 1634851

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```

Met Cys Thr Gly Lys Cys Ala Arg Cys Val Gly Leu Ser Leu Ile Thr
 1             5             10             15
Leu Cys Leu Val Cys Ile Val Ala Asn Ala Leu Leu Leu Val Pro Asn
 20             25             30
Gly Glu Thr Ser Trp Thr Asn Thr Asn His Leu Ser Leu Gln Val Trp
 35             40             45
Leu Met Gly Gly Phe Ile Gly Gly Gly Leu Met Val Leu Cys Pro Gly
 50             55             60
Ile Ala Ala Val Arg Ala Gly Gly Lys Gly Cys Cys Gly Ala Gly Cys
 65             70             75             80
Cys Gly Asn Arg Cys Arg Met Leu Arg Ser Val Phe Ser Ser Ala Phe
 85             90             95

```

Gly Val Leu Gly Ala Ile Tyr Cys Leu Ser Val Ser Gly Ala Gly Leu  
 100 105 110  
 Arg Asn Gly Pro Arg Cys Leu Met Asn Gly Glu Trp Gly Tyr His Phe  
 115 120 125  
 Glu Asp Thr Ala Gly Ala Tyr Leu Leu Asn Arg Thr Leu Trp Asp Arg  
 130 135 140  
 Cys Glu Ala Pro Pro Arg Val Val Pro Trp Asn Val Thr Leu Phe Ser  
 145 150 155 160  
 Leu Leu Val Ala Ala Ser Cys Leu Glu Ile Val Leu Cys Gly Ile Gln  
 165 170 175  
 Leu Val Asn Ala Thr Ile Gly Val Phe Cys Gly Asp Cys Arg Lys Lys  
 180 185 190  
 Gln Asp Thr Pro His  
 195

## (2) INFORMATION FOR SEQ ID NO:2:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 711 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (vii) IMMEDIATE SOURCE:

- (A) LIBRARY: COLNNOT09  
 (B) CLONE: 1634851

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

CGGCTCGAGC	GGCTCGAGCC	TGACACCTCA	CCATGTGTAC	GGGAAAATGT	GCCCGCTGTG	60
TGGGGCTCTC	CCTCATACC	CTCTGCCTCG	TCTGCATTGT	GGCCAACGCC	CTCCTGCTGG	120
TACCTAATGG	GGAGACCTCC	TGGACCAACA	CCAACCATCT	CAGCTTGCAA	GTCTGGCTCA	180
TGGCGGGCTT	CATTGGCGGG	GGCCTAATGG	TACTGTGTCC	AGGGATTGCA	GCCGTTGCGG	240
CAGGGGGCAA	GGGCTGCTGT	GGTGCTGGGT	GCTGTGGAAA	CCGCTGCAGG	ATGCTGCGCT	300
CGGTCTTCTC	CTCGGCGTTC	GGGGTGCTTG	GTGCCATCTA	CTGCCTCTCG	GTGTCTGGAG	360
CTGGGCTCCG	AAATGGACCC	AGATGCTTAA	TGAACGGCGA	GTGGGGCTAC	CACTTCGAAG	420
ACACCGCGGG	AGCTTACTTG	CTCAACCGCA	CTCTATGGGA	TGGGTGCGAG	GCGCCCCCTC	480
GCGTGGTCCC	CTGGAATGTG	ACGCTCTTCT	CGCTGCTGGT	GGCCGCCTCC	TGCCTGGAGA	540
TAGTACTGTG	TGGGATCCAG	CTGGTGAACG	CGACCATTTG	TGTCTTCTGC	GGCGATTGCA	600
GGAAAAAACA	GGACACACCT	CAGTGAGGCT	CCACTGACCG	CCGGGTACAC	CCTGCTCCTT	660
CCTGGACGCT	CACTCCCTTG	CTCGCTAGAA	TAACTGCTT	TGCGCTCTCT	T	711

## (2) INFORMATION FOR SEQ ID NO:3:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 202 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (vii) IMMEDIATE SOURCE:

- (A) LIBRARY: GenBank  
 (B) CLONE: 186804

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Met	Cys	Tyr	Gly	Lys	Cys	Ala	Arg	Cys	Ile	Gly	His	Ser	Leu	Val	Gly
1			5						10					15	
Leu	Ala	Leu	Leu	Cys	Ile	Ala	Ala	Asn	Ile	Leu	Leu	Tyr	Phe	Pro	Asn
		20						25					30		
Gly	Glu	Thr	Lys	Tyr	Ala	Ser	Glu	Asn	His	Leu	Ser	Arg	Phe	Val	Trp
		35				40					45				
Phe	Phe	Ser	Gly	Ile	Val	Gly	Gly	Gly	Leu	Leu	Met	Leu	Leu	Pro	Ala
	50					55					60				
Phe	Val	Phe	Ile	Gly	Leu	Glu	Gln	Asp	Asp	Cys	Cys	Gly	Cys	Cys	Gly
	65				70				75					80	
His	Glu	Asn	Cys	Gly	Lys	Arg	Cys	Ala	Met	Leu	Ser	Ser	Val	Leu	Ala
			85						90					95	

Ala Leu Ile Gly Ile Ala Gly Ser Gly Tyr Cys Val Ile Val Ala Ala  
 100 105 110  
 Leu Gly Leu Ala Glu Gly Pro Leu Cys Leu Asp Ser Leu Gly Gln Trp  
 115 120 125  
 Asn Tyr Thr Phe Ala Ser Thr Glu Gly Gln Tyr Leu Leu Asp Thr Ser  
 130 135 140  
 Thr Trp Ser Glu Cys Thr Glu Pro Lys His Ile Val Glu Trp Asn Val  
 145 150 155 160  
 Ser Leu Phe Ser Ile Leu Leu Ala Leu Gly Gly Ile Glu Phe Ile Leu  
 165 170 175  
 Cys Leu Ile Gln Val Ile Asn Gly Val Leu Gly Gly Ile Cys Gly Phe  
 180 185 190  
 Cys Cys Ser His Gln Gln Gln Tyr Asp Cys  
 195 200

## (2) INFORMATION FOR SEQ ID NO:4:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 202 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (vii) IMMEDIATE SOURCE:

- (A) LIBRARY: GenBank  
 (B) CLONE: 476343

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Cys Tyr Val Lys Cys Ala Arg Tyr Ile Gly Tyr Ser Leu Val Trp  
 1 5 10 15  
 Ala Ala Val Phe Cys Ile Val Ala Asn Ala Leu Leu Tyr Phe Pro Asn  
 20 25 30  
 Gly Glu Thr Lys Tyr Ala Thr Glu Asp His Leu Ser Arg Phe Val Trp  
 35 40 45  
 Tyr Phe Ala Gly Ile Val Gly Gly Leu Leu Met Leu Leu Pro Ala  
 50 55 60  
 Phe Val Phe Ile Gly Met Asp Glu Glu Asp Cys Cys Gly Cys Cys Gly  
 65 70 75 80  
 Tyr Glu Asn Tyr Gly Lys Arg Cys Ser Met Leu Ser Ser Val Leu Ala  
 85 90 95  
 Ala Leu Ile Gly Ile Val Gly Ser Ala Tyr Cys Val Ile Val Ala Ser  
 100 105 110  
 Leu Gly Leu Ala Glu Gly Pro Lys Cys Ser Asp Ala His Gly Val Trp  
 115 120 125  
 Asn Tyr Thr Phe Ala Ser Thr Glu Gly Gln Tyr Leu Leu Asn Ser Ser  
 130 135 140  
 Met Trp Ser Lys Cys Tyr Glu Pro Lys His Ile Val Glu Trp His Val  
 145 150 155 160  
 Thr Leu Phe Ser Ile Leu Leu Ala Phe Ala Ala Val Glu Phe Ile Leu  
 165 170 175  
 Cys Leu Ile Gln Val Ile Asn Gly Met Leu Gly Gly Leu Cys Gly Tyr  
 180 185 190  
 Cys Cys Ser Arg Gln Gln Gln Tyr Asn Cys  
 195 200

What is claimed is:

1. A substantially purified human tumor-associated antigen comprising the amino acid sequence of SEQ ID NO:1 or fragments thereof.
2. An isolated and purified polynucleotide sequence encoding the human tumor-associated antigen of claim 1.
3. A polynucleotide sequence which hybridizes under stringent conditions to the polynucleotide sequence of claim 2.
4. A composition comprising the polynucleotide sequence of claim 2.
5. An isolated and purified polynucleotide sequence comprising SEQ ID NO:2 or variants thereof.
6. A polynucleotide sequence which is complementary to the polynucleotide sequence of claim 2 or variants thereof.
7. A composition comprising the polynucleotide sequence of claim 6.
8. An expression vector containing the polynucleotide sequence of claim 2.
9. A host cell containing the vector of claim 8.
10. A method for producing a polypeptide comprising the amino acid sequence of SEQ ID NO:1, or fragments thereof, the method comprising the steps of:
  - a) culturing the host cell of claim 9 under conditions suitable for the expression of the polypeptide; and
  - b) recovering the polypeptide from the host cell culture.
11. A pharmaceutical composition comprising a substantially purified human tumor-associated antigen having the amino acid sequence of SEQ ID NO:1 in conjunction with a suitable pharmaceutical carrier.
12. A purified antibody which binds specifically to the polypeptide of claim 1.
13. A purified agonist which modulates the activity of the polypeptide of claim 1.
14. A purified antagonist which decreases the effect of the polypeptide of claim 1.
15. A method for stimulating cell proliferation comprising adding to a cell an effective amount of the protein of claim 1.
16. A pharmaceutical composition comprising the antagonist of claim 14 in conjunction with a suitable pharmaceutical carrier.
17. A method for treating or preventing a cancer comprising administering to a subject in need of such treatment the pharmaceutical composition of claim 16.

18. A method for treating or preventing inflammation comprising administering to a subject in need of such treatment the pharmaceutical composition of claim 16.

19. A method for detecting a polynucleotide which encodes the human tumor-associated antigen in a biological sample comprising the steps of:

- 5           a)     hybridizing the polynucleotide of claim 6 to nucleic acid material of a biological sample, thereby forming a hybridization complex; and
- b)     detecting said hybridization complex, wherein the presence of said complex correlates with the presence of a polynucleotide encoding the human tumor-associated antigen in said biological sample.

10



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9	18	27	36	45	54
NCG GCT CGA GCG GCT CGA GCC TGA CAC CTC ACC ATG TGT ACG GGA AAA TGT GCC					
			M C T G	G G K C	A
63	72	81	90	99	108
CGC TGT GTG GGG CTC TCC CTC ATT ACC CTC TGC CTC GTG ATT GTG GCC AAC					
R C V G L S L I T L C L V C I V A N					
117	126	135	144	153	162
GCC CTC CTG CTG GTA CCT AAT GGG GAG ACC TCC TGG ACC AAC ACC AAC CAT CTC					
A L L L V P N G E T S W T N T N H L					
171	180	189	198	207	216
AGC TTG CAA GTC TGG CTC ATG GGC GGC TTC ATT GGC GGC CTA ATG GTA CTG					
S L Q V W L M G G F I G G L M V L					
225	234	243	252	261	270
TGT CCA GGG ATT GCA GCC GTT CGG GCA GGC GGC AAG GGC TGC TGT GGT GCT GGG					
C P G I A A V R A G G K G C C G A G					
279	288	297	306	315	324
TGC TGT GGA AAC CGC TGC AGG ATG CTG CGC TCG GTC TTC TCC TCG GCG TTC GGG					
C C G N R C R M L R S V F S S A F G					
333	342	351	360	369	378
GTG CTT GGT GCC ATC TAC TGC CTC TCG GTG TCT GGA GCT GGG CTC CGA AAT GGA					
V L G A I Y C L S V S G A G L R N G					

FIGURE 1A

387 CCC AGA TGC TTA ATG AAC GGC GAG TGG GGC TAC CAC TTC GAA GAC ACC GCG GGA 432  
 P R C L M N G E W G Y H F E D T A G  
 441 GCT TAC TTG CTC AAC CGC ACT CTA TGG GAT CGG TGC TGG GCG CCC CCT CGC GTG 486  
 A Y L L N R T L W D R C E A P P R V  
 495 GTC CCC TGG AAT GTG ACG CTC TTC TCG CTG CTG GCG GCC TCC TGC CTG GAG 540  
 V P W N V T L F S L L V A A S C L E  
 549 ATA GTA CTG TGT GGT GGC ATC CAG CTG GTG AAC GCG ACC ATT GGT GTC TTC TGC GGC 594  
 I V L C G I Q L V N A T I G V F C G  
 603 GAT TGC AGG AAA CAG GAC GAC ACA CCT CAC TGA GGC TCC ACT GAC CGC CGG GTT 648  
 D C R K K Q D T P H  
 657 ACA CCT GCT CCT TCC TGG ACG CTC ACT CCC TTG CTC GCT AGA ATA AAC TGC TTT 702  
 711 GCG CTC TCT T

FIGURE 1B

1	M	C	T	G	K	C	A	R	C	V	G	L	S	L	I	T	L	C	L	V	C	I	V	A	N	A	L	L	V	P	N	G	E	T	S	W	T	N	T	1634851		
1	M	C	Y	G	K	C	A	R	C	I	G	H	S	L	V	G	L	A	L	L	C	I	A	N	I	L	L	Y	F	F	P	N	G	E	T	K	Y	A	S	E	GI 186804	
1	M	C	Y	V	K	C	A	R	Y	I	G	Y	S	L	V	W	A	A	V	F	C	I	V	A	N	A	L	L	Y	F	F	P	N	G	E	T	K	Y	A	T	E	GI 476343
41	N	H	L	S	L	Q	V	W	L	M	G	G	F	I	G	G	G	L	M	V	L	C	P	G	I	A	A	V	R	A	G	G	K	G	C	C	G	A	G	C	1634851	
41	N	H	L	S	R	F	V	W	F	F	S	G	I	V	G	G	G	L	L	M	L	L	P	A	F	V	F	I	G	L	E	Q	D	D	C	C	G	-	-	C	GI 186804	
41	D	H	L	S	R	F	V	W	Y	F	A	G	I	V	G	G	G	L	L	M	L	L	P	A	F	V	F	I	G	M	D	E	E	D	C	C	G	-	-	C	GI 476343	
81	C	G	-	-	-	-	N	R	C	R	M	L	R	S	V	F	S	S	A	F	G	V	L	G	A	I	Y	C	L	S	V	S	G	A	G	L	R	N	G	1634851		
79	C	G	H	E	N	C	G	K	R	C	A	M	L	S	S	V	L	A	A	L	I	G	I	A	G	S	G	Y	C	V	I	V	A	A	L	G	L	A	E	G	GI 186804	
79	C	G	Y	E	N	Y	G	K	R	C	S	M	L	S	S	V	L	A	A	L	I	G	I	V	G	S	A	Y	C	V	I	V	A	S	L	G	L	A	E	G	GI 476343	
116	P	R	C	L	-	M	N	G	E	W	G	Y	H	F	E	D	T	A	G	A	Y	L	L	N	R	T	L	W	D	R	C	E	A	P	P	R	V	V	P	W	1634851	
119	P	L	C	L	D	S	L	G	Q	W	N	Y	T	F	A	S	T	E	G	Q	Y	L	L	D	T	S	T	W	S	E	C	T	E	P	K	H	I	V	E	W	GI 186804	
119	P	K	C	S	D	A	H	G	V	W	N	Y	T	F	A	S	T	E	G	Q	Y	L	L	N	S	S	M	W	S	K	C	Y	E	P	K	H	I	V	E	W	GI 476343	
155	N	V	T	L	F	S	L	L	V	A	A	S	C	L	E	I	V	L	C	G	I	Q	L	V	N	A	T	I	G	V	F	C	G	D	C	-	R	K	K	Q	1634851	
159	N	V	S	L	F	S	I	L	L	A	L	G	G	I	E	F	I	L	C	L	I	Q	V	I	N	G	V	L	G	G	I	C	G	F	C	C	S	H	Q	Q	GI 186804	
159	H	V	T	L	F	S	I	L	L	A	F	A	V	E	F	I	L	C	L	I	Q	V	I	N	G	M	L	G	G	L	C	G	Y	C	C	S	R	Q	Q	GI 476343		
194	D	T	P	H																																				1634851		
199	Q	Y	D	C																																				GI 186804		
199	Q	Y	N	C																																				GI 476343		

FIGURE 2

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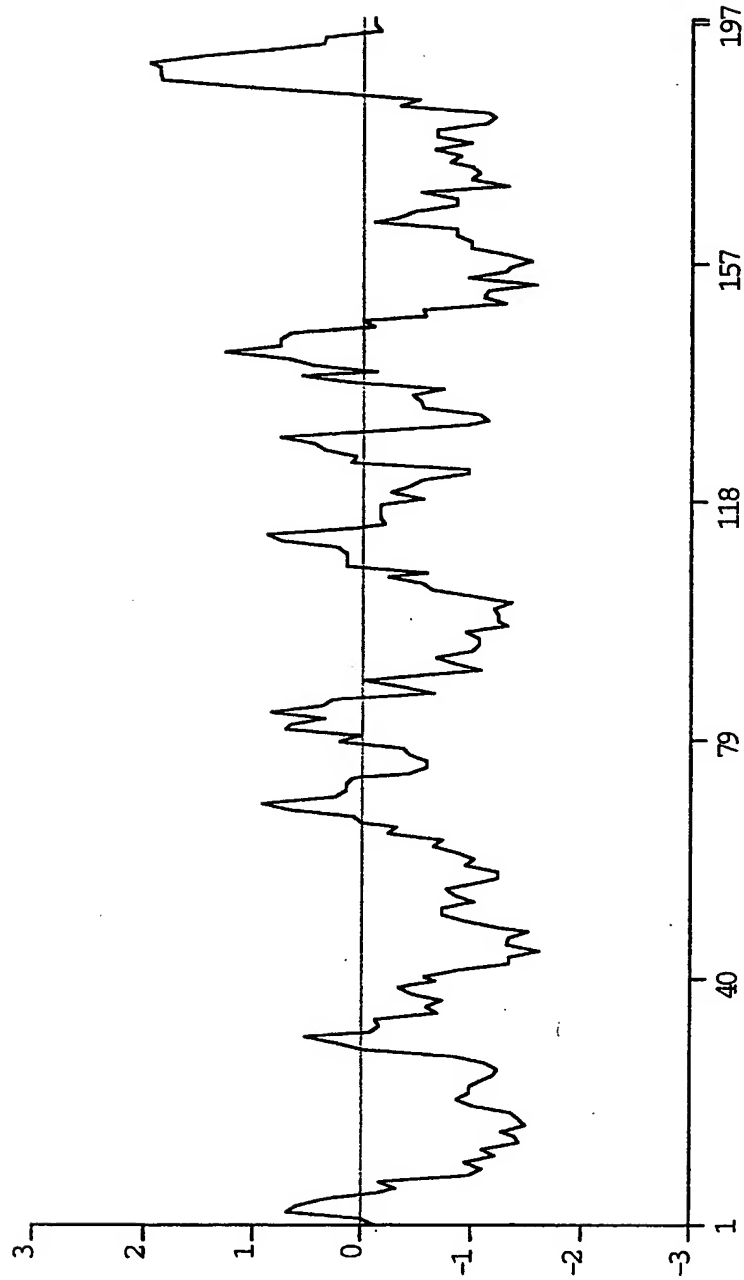


FIGURE 3A

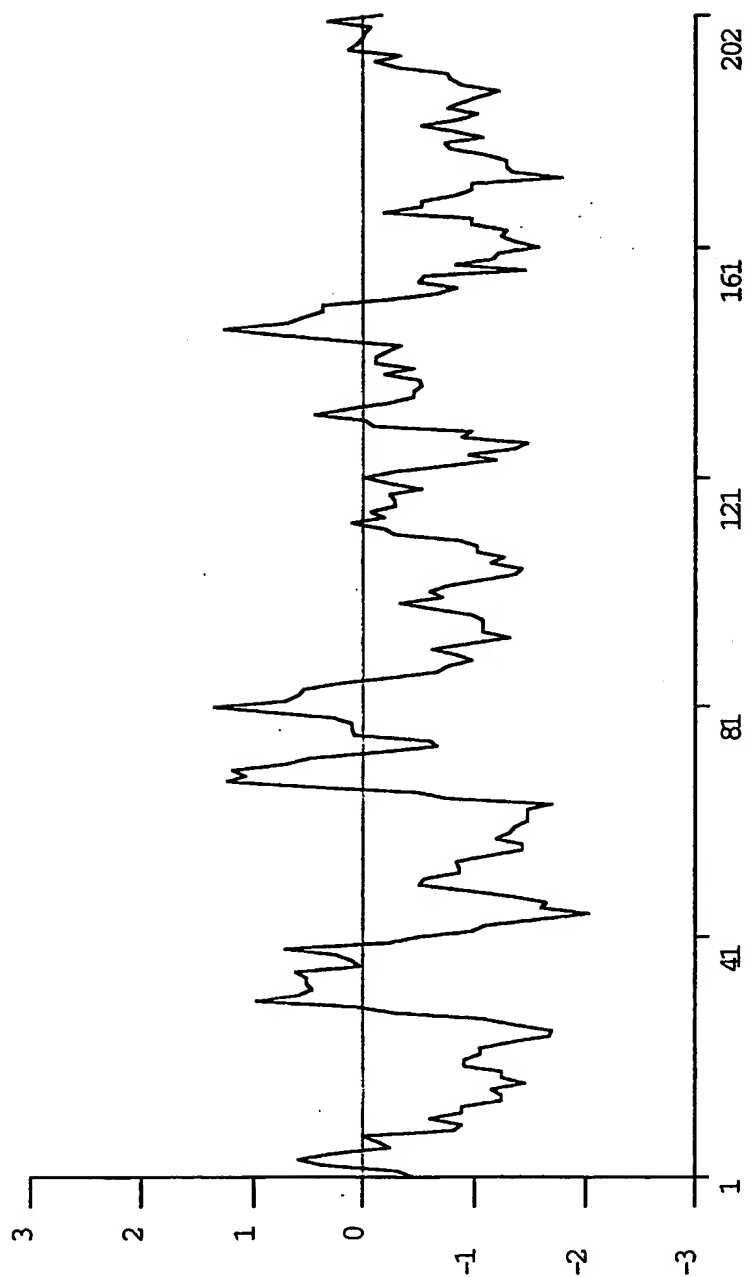


FIGURE 3B

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 98/09876

## A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C07K 14/705, A61K 38/17, C07K 16/30

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: A61K, C07K, C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	Dialog Information Services, File 34, SciSearch, Dialog accession no. 06529072, "Identification of a new tumour-associated antigen TM4SF5 and its expression in human cancer", Gene, 1998, V208, N1 (FEB 16), P25-30 --	1-19
P,X	EMBL, Databas GenBank/DBJ, accession no. AF027204, Mueller-Pillasch F. et al: "Homo sapiens putative tetraspan transmembrane protein L6H (TM4SF5)", & 1997-11-18 --	1-19
X	US 5597707 A (JOHN MARKEN ET AL), 28 January 1997 (28.01.97) -----	1-19

☐ Further documents are listed in the continuation of Box C.☒ See patent family annex.

## \* Special categories of cited documents:

\*A\* document defining the general state of the art which is not considered to be of particular relevance

\*E\* earlier document but published on or after the international filing date

\*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

\*O\* document referring to an oral disclosure, use, exhibition or other means

\*P\* document published prior to the international filing date but later than the priority date claimed

\*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

\*X\* document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

\*Y\* document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

\*&amp;\* document member of the same patent family


Date of the actual completion of the international search

24 August 1998

Date of mailing of the international search report

15. 09. 1998

Name and mailing address of the ISA/



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PATRICK ANDERSSON

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 98/ 09876

## Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
Remark: Although claims 17-18 are directed to a method of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.  
(c.f. PCT Rule 39.1(iv))
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (1)) (July 1992)

International application No.  
PCT/US 98/09876

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5597707 A	28/01/97	NONE	